11-7-05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: De Jong et al.	Art Unit	: 1636
Patent No.	: 6,936,469 B2	Examiner	: Daniel M. Sullivan
Issue Date	: August 30, 2005	Cust. No.	: 20985
Serial No.	: 09/815,981	Conf. No.	: 7622
Filed	: March 22, 2001		
Title	: METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF		

ATTN.: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION PURSUANT TO 37 C.F.R. §§ 1.322 AND 1.323

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.322 and 1.323, the patentee respectfully requests that a Certificate of Correction be issued for the above-referenced patent to correct the following errors:

IN THE SPECIFICATION:

At page 21, line 10, please replace the formula "$CO_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$" with ——$C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$——.

At page , line please replace the phrase "SEQ ID NO. 16." with ——SEQ ID NO. 16 of PCT Application Publication No. WO97/40183.——

IN THE CLAIMS:

Please amend claims 1, 10, 15, and 16 as follows (deletions are [[double bracketed]], additions are underlined).

1. A method for detecting or determining delivery and expression of <u>a</u> nucleic acid introduced into a cell comprising:

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
"Express Mail" Mailing Label Number EV 471534295
Date of Deposit November 3, 2005
I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to: Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450.

Stephanie L. Seidman

OIPE IAP19
NOV 0 3 2005
PATENT & TRADEMARK OFFICE

/

Attorney's Docket No.: 17084-018002/416B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: De Jong et al.	Art Unit	: 1636
Patent No.	: 6,936,469 B2	Examiner	: Daniel M. Sullivan
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Attn: Certificate of Correction Branch
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TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith are a Request for Certificate of Correction Pursuant to 37 C.F.R. §§ 1.322, Certificate of Correction Form PTO-1050 (2 pages), documents as evidence, a check in the amount of $100.00, and a return postcard for filing in connection with the above-identified application.

☒ The Commissioner is hereby authorized to charge any fees that may be due in connection with this paper or with this application during its entire pendency to Deposit Account No. 06-1050. A duplicate of this sheet is enclosed.

Respectfully submitted,

Stephanie L. Seidman
Reg. No. 33,779

Attorney Docket No. 17084-018002/416B
Address all correspondence to:
Stephanie L. Seidman
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130
Telephone: (858) 678-5070
Facsimile: (202) 626-7796
email: seidman@fr.com

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Stephanie L. Seidman

NOV 1 7 2005

Attorney's Docket No.: 17084-018002/416B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	De Jong et al.	Art Unit	:	1636
Patent No.	:	6,936,469 B2	Examiner	:	Daniel M. Sullivan
Issue Date	:	August 30, 2005	Cust. No.	:	20985
Serial No.	:	09/815,981	Conf. No.	:	7622
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Stephanie L. Seidman

introducing labelled large nucleic acid molecules that encode a reporter gene into cells, wherein the labelled large nucleic acid is labelled with a label selected from the group consisting of a nucleotide analog, a nucleoside analog and a ribonucleoside analog;

detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and

measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.

10. The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), ~~2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N.N-dimethyl-1-propanaminiumtrifluoroacetate~~ 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4.4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$, ~~$C_{100}H_{206}N_{12}O_4S_26.8CF3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CF_3CO_2H$, $C_{43}H_{88}N_4O_22CF_3CO_2H$,~~ $C_{100}H_{206}N_{12}O_4S_2.8CF3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

15. The method of claim [[1]]14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. The method of claim [[1]]14, wherein the cells are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

introducing labelled large nucleic acid molecules that encode a reporter gene into cells, wherein the labelled large nucleic acid is labelled with a label selected from the group consisting of a nucleotide analog, a nucleoside analog and a ribonucleoside analog;

detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and

measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.

10. The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N[[.]],N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4.4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{100}H_{206}N_{12}O_4S_2[[6]].8CF3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3[[CF_3]]CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

15. The method of claim [[1]]14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. The method of claim [[1]]14, wherein the cells are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

REMARKS

A Certificate of Correction (Form PTO-1050) incorporating the above changes are included with this Request. A check is enclosed to cover the required fee. If is determined that the fee amount is incorrect or if the check is missing, the Office is hereby authorized to charge the fee to Deposit Account No. 06-1050.

This Certificate of Correction seeks to correct a typographical error introduced by the PTO in the specification. At page 21, line 10, the chemical formula "$CO_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$" should read as "$C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$", as originally filed.

This Certificate of Correction also seeks to correct the specification at page 37, line 37, by replacing the phrase "SEQ ID NO. 16." with the phrase "SEQ ID NO. 16 of PCT Application Publication No. WO97/40183." This correction further clarifies the reference to SEQ ID NO. 16, which is of PCT Application Publication No. WO97/40183. Basis for this correction can be found on page 37, line 34, which references PCT Application Publication No. WO97/40183. No new matter has been added.

This Certificate of Correction also seeks to correct errors in the claims introduced by the PTO. In claim 1, the article "a" was deleted by the PTO. This typographical error was previously amended in an Amendment, filed by the applicant on June 16, 2003. Acknowledgement of the Amendment was given as part of paper 22 mailed on September 22, 2003. As evidence, a copy of the Amendment with the returned date-stamped postcard dated June 16, 2003 is provided herewith. In claim 10, the chemical name "2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N.N-dimethyl-1-propanaminiumtrifluoroacetate" should read as "2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate". The period "." occurring within the chemical name has been amended to a comma "," to correct this error. Also in claim 10, the chemical formula "$C_{162}H_{330}N_{22}O_9.13CF_3CF_3CO_2H$" should read as "$C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$". The chemical formula has been amended to delete the second occurrence of "CF_3". Also in claim 10, the chemical formula "$C_{43}H_{88}N_4O_22CF_3CO_2H$" should read as "$C_{43}H_{88}N_4O_2.2CF_3CO_2H$". The chemical formula has been amended to add the omitted period.

In the Amendment filed by the applicant on June 16, 2003, the chemical formula "$C_{100}H_{206}N_{12}O_4S_2.8CF3CO_2H$" in claim 10, was inadvertently amended to read as

"$C_{100}H_{206}N_{12}O_4S_26$•8CF3$CO_2H$". This Certificate of Correction seeks to correct the chemical formula by deleting the number "6" such that the chemical formula now reads as "$C_{100}H_{206}N_{12}O_4S_2$.8CF3$CO_2H$".

This Certificate of Correction further seeks to correct the errors in claim dependencies of claims 15 and 16 introduced by the PTO. In claim 15, the claim dependency has been corrected from claim 1 to claim 14. Basis for this correction can be found in the Preliminary Amendment filed by the applicant on May 25, 2001, wherein the claim dependency of claim 16 was amended from claim 15 to claim 14. As evidence, a copy of the Preliminary Amendment with the returned date-stamped postcard dated May 25, 2001, is provided herewith. In claim 16, the claim dependency has been corrected from claim 1 to claim 14. Basis for this correction can be found in claim 16 as originally filed.

* * * * *

This Certificate of Correction seeks to amend these errors in the Specification and Claims introduced by the Patent and Trademark Office and the applicant. These changes do not constitute new matter. Patentee respectfully requests correction of errors by issuance of a Certificate of Correction.

Respectfully submitted,

Dated: November 3, 2005

Stephanie L. Seidman
Reg. No. 33,779

Attorney Docket No. 17084-018002/416B
Address all correspondence to:
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Fish & Richardson P.C.
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email: seidman@fr.com

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10559379.doc

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,936,469

DATED : AUGUST 30, 2005

INVENTOR(S) : GARY DE JONG AND SANDRA LOUISE VANDERBYL

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

IN THE SPECIFICATION:

At column 12, line 9, the formula "$CO_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$" should read as ––$C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$––.

At column 21, line 29, the phrase "SEQ ID NO. 16." should read as ––SEQ ID NO. 16 of PCT Application Publication No. WO97/40183.––

IN THE CLAIMS:
Claims 1, 10, 15, and 16 should read as follows:

1. A method for detecting or determining delivery and expression of a nucleic acid introduced into a cell comprising:

introducing labelled large nucleic acid molecules that encode a reporter gene into cells, wherein the labelled large nucleic acid is labelled with a label selected from the group consisting of a nucleotide analog, a nucleoside analog and a ribonucleoside analog;

detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and

measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.

MAILING ADDRESS OF SENDER:

PATENT NO. 6,936,469

Stephanie Seidman
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,936,469

DATED : AUGUST 30, 2005

INVENTOR(S) : GARY DE JONG AND SANDRA LOUISE VANDERBYL

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

10. The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4.4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{100}H_{206}N_{12}O_4S_2.8CF3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

15. The method of claim 14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. The method of claim 14, wherein the cells are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

MAILING ADDRESS OF SENDER: PATENT NO. 6,936,469

Stephanie Seidman
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130

OIPE IAP19
NOV 0 3 2005
PATENT & TRADEMARK OFFICE

THE STAMP OF THE U.S. PATENT AND TRADEMARK OFFICE AFFIXED HERETO WILL BE EVIDENCE OF RECEIPT OF THE FOLLOWING SENT VIA FIRST CLASS (PRIORITY) MAIL ON MAY 23,2001:

CLIENT #: 24601-416B SLS:RHT:kmf
ENCLOSURES: TRANSMITTAL LETTER (in duplicate); CHANGE OF ADDRESS FORM (in duplicate); PRELIMINARY AMENDMENT (15 pages); ATTACHMENT TO PRELIMINARY AMENDMENT (14 pages); and RETURN POSTCARD.
APPLICANT: DE JONG ET AL.
APP. NO: 09/815,981 FILED: MARCH 22, 2001
FOR: METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF.

PLEASE STAMP HERE (THANK YOU):

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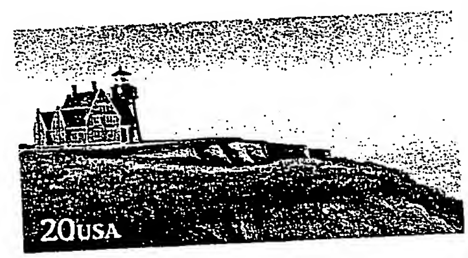

Stephanie L. Seidman, Ph.D.
Heller Ehrman White & McAuliffe LLP
4250 Executive Square, 7th Floor
La Jolla, CA 92037-9103

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PLEASE STAMP HERE (THANK YOU):

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PATENT & TRADEMARK OFFICE

OIPE JC174
MAY 2 5 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: De Jong *et al.*

Serial No.: 09/815,981

Filed: March 22, 2001

For: METHOD FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF

Art Unit: Unassigned

Examiner: Unassigned

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Commissioner for Patents
Washington, D.C. 20231, on this date.

05/23/01
Date

Kelly Fischer
Kelly Fischer

TRANSMITTAL LETTER

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Transmitted herewith are a Preliminary Amendment and a Change of Address Notification for filing in connection with the above-identified application.

☒ The Commissioner is hereby authorized to charge any fee, including any submitted herewith if the attached check(s) is in the wrong amount or otherwise improper or missing, that may be due in connection with this and the attached papers, or with this application during its entire pendency to or to credit any overpayment to Deposit Account No. 50-1213. A duplicate of this sheet is enclosed.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket No. 24601-416B

Address all correspondence to:
Stephanie L. Seidman, Esq.
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Telephone: (858) 450-8400
Facsimile: (858) 587-5360
E-mail: sseidman@HEWM.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: De Jong *et al.*

Serial No.: 09/815,981

Filed: March 22, 2001

For: METHOD FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF

Art Unit: Unassigned

Examiner: Unassigned

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Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket No. 24601-416B

Address all correspondence to:
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4350 La Jolla Village Drive, 6th Floor
San Diego, California 92122-1246
Telephone: (858) 450-8400
Facsimile: (858) 587-5360
E-mail: sseidman@HEWM.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: De Jong *et al.*

Serial No.: 09/815,981

Filed: March 22, 2001

For: METHOD FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF

Art Unit: Unassigned
Examiner: Unassigned

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05/23/01
Date

Kelly Fischer
Kelly Fischer

CHANGE OF ADDRESS

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please send all correspondence for this application to:

STEPHANIE L. SEIDMAN, ESQ.
Heller Ehrman White & McAuliffe
4350 La Jolla Village Drive, 6th Fl.
San Diego, CA 92122-1246

The telephone number is (858) 450-8400, and the facsimile number is (858) 587-5360.

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Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket 24601-416B

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Serial No.: 09/815,981

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Art Unit: Unassigned
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Kelly Fischer

CHANGE OF ADDRESS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: De Jong *et al.*

Serial No.: 09/815,981

Filed: March 22, 2001

For: *METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF*

Art Unit: Unassigned

Examiner: Unassigned

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Commissioner for Patents
Washington, D.C. 20231, on this date.

05/23/01
Date

Kelly Fischer
Kelly Fischer

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination of the above-captioned patent application, please amend the application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows (a marked-up copy of the amended specification is attached to this Amendment):

Please replace the paragraph on page 5, lines 3-7, with the following paragraph:

Included among the nucleic acid molecules that may be delivered into cells using the methods provided herein are artificial chromosomes, satellite DNA-based artificial chromosomes (SATACs, herein referred to as ACes) and natural chromosomes or fragments of any of these chromosomes.

Please replace the paragraph on page 8, lines 11-30, with the following paragraph:

In particular embodiments, the methods of monitoring delivery and expression of a nucleic acid molecule include the steps of: introducing labelled nucleic acid molecules that encode a reporter gene into cells;

detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined. The labelled cells can be detected, for example, by flow cytometry, fluorimetry, cell imaging or fluorescence spectroscopy. The label, for example, can be iododeoxyuridine (IdU or IdUrd) or bromodeoxyuridine (BrdU), the reporter gene, for example, can be one that encodes fluorescent protein, enzyme, such as a luciferase, or antibody. The delivered nucleic acid molecules include, but are not limited to, RNA, including ribozymes, DNA, including naked DNA and chromosomes, plasmids, chromosome fragments, typically containing at least one gene or at least 1 Kb, naked DNA, or natural chromosomes. The method is exemplified herein by determining delivery and expression of artificial chromosome expression systems (ACes). Any types of cells, eukaryotic and prokaryotic, including cell lines, primary cell lines, plant cells, and animal cells, including stem cells, embryonic cells, and other cells into which delivery of a nucleic acid molecule can occur is contemplated.

Please replace the paragraph beginning on page 13, line 26, through page 14, line 7, with the following paragraph:

As used herein, cationic compounds are compounds that have polar groups that are positively charged at or around physiological pH. These compounds facilitate delivery of nucleic acid molecules into cells; it is thought this is achieved by virtue of their ability to neutralize the electrical charge of nucleic acids. Exemplary cationic compounds include, but are not limited to, cationic lipids or cationic polymers or mixtures thereof, with or without neutral lipids, polycationic lipids, non-liposomal forming lipids, ethanolic cationic lipids and cationic amphiphiles. Contemplated cationic compounds also include activated dendrimers, which are spherical cationic polyamidoamine polymers with a defined spherical architecture of charged amino groups which branch

from a central core and which can interact with the negatively charged phosphate groups of nucleic acids (e.g., starburst dendrimers).

Please replace the paragraph beginning on page 15, line 18, through page 16, line 4, with the following paragraph:

As used herein, gene therapy involves the transfer or insertion of nucleic acid molecules, and, in particular, large nucleic acid molecules, into certain cells, which are also referred to as target cells, to produce specific gene products that are involved in correcting or modulating diseases or disorders. The nucleic acid is introduced into the selected target cells in a manner such that the nucleic acid is expressed and a product encoded thereby is produced. Alternatively, the nucleic acid may in some manner mediate expression of DNA that encodes a therapeutic product. This product may be a therapeutic compound, which is produced in therapeutically effective amounts or at a therapeutically useful time. It may also encode a product, such as a peptide or RNA, that in some manner mediates, directly or indirectly, expression of a therapeutic product. Expression of the nucleic acid by the target cells within an organism afflicted with a disease or disorder thereby provides a way to modulate the disease or disorder. The nucleic acid encoding the therapeutic product may be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

Please replace the paragraph beginning on page 16, line 24, through page 17, line 8, with the following paragraph:

As used herein, a reporter gene construct is a DNA molecule that includes a reporter gene operatively linked to a transcriptional control sequence. The transcriptional control sequences include the promoter and other optional regulatory regions, such as enhancer sequences, that modulate the activity of the promoter, or control sequences that modulate the activity or efficiency of the RNA polymerase that recognizes the promoter, or control sequences that are

recognized by effector molecules, including those that are specifically induced by interaction of an extracellular signal with a cell surface protein. For example, modulation of the activity of the promoter may be effected by altering the RNA polymerase binding to the promoter region, or, alternatively, by interfering with initiation of transcription or elongation of the mRNA. Such sequences are herein collectively referred to as transcriptional control elements or sequences. In addition, the construct may include sequences of nucleotides that alter translation of the resulting mRNA, thereby altering the amount of reporter gene product.

Please replace the paragraphs beginning on page 19, line 24, through page 20, line 18, with the following paragraphs:

Cationic Compounds

Cationic compounds for use in the methods provided herein are available commercially or can be synthesized by those of skill in the art. Any cationic compound may be used for delivery of nucleic acid molecules, such as DNA, into a particular cell type using the provided methods. One of skill in the art by using the provided screening procedures can readily determine which of the cationic compounds are best suited for delivery of specific nucleic acid molecules, such as DNA, into a specific target cell type.

(a) Cationic Lipids

Cationic lipid reagents can be classified into two general categories based on the number of positive charges in the lipid headgroup; either a single positive charge or multiple positive charges, usually up to 5. Cationic lipids are often mixed with neutral lipids prior to use as delivery agents. Neutral lipids include, but are not limited to, lecithins; phosphatidylethanolamine; phosphatidylethanolamines, such as DOPE (dioleoylphosphatidylethanolamine), DPPE (dipalmitoylphosphatidylethanolamine), POPE (palmitoyloleoylphosphatidylethanolamine) and distearoylphosphatidylethanolamine; phosphatidylcholine; phosphatidylcholines, such as DOPC (dioleoylphosphatidylcholine), DPPC

(dipalmitoylphosphatidylcholine), POPC (palmitoyloleoylphosphatidylcholine) and distearoylphosphatidylcholine; fatty acid esters; glycerol esters; sphingolipids; cardiolipin; cerebrosides; and ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3βOH-sterols.

Please replace the paragraph beginning on page 20, line 24, through page 25, line 18, with the following paragraph:

Examples of cationic lipid compounds include, but are not limited to: Lipofectin (Life Technologies, Inc., Burlington, Ont.)(1:1 (w/w) formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dioleoylphosphatidylethanolamine (DOPE); LipofectAMINE (Life Technologies, Burlington, Ont., see U.S. Patent No. 5,334,761) (3:1 (w/w) formulation of polycationic lipid 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) and dioleoylphosphatidylethanolamine (DOPE), LipofectAMINE PLUS (Life Technologies, Burlington, Ont. see U.S. Patent Nos. 5,334,761 and 5,736,392; see, also U.S. Patent No. 6,051,429) (LipofectAmine and Plus reagent), LipofectAMINE 2000 (Life Technologies, Burlington, Ont.; see also International PCT application No. WO 00/27795) (Cationic lipid), Effectene (Qiagen, Inc., Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, Inc., San Luis Obispo, CA) (ethanolic cationic lipids numbers 1 through 12: $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$); Cytofectene (Bio-Rad, Hercules, CA) (mixture of a cationic lipid and a neutral lipid), GenePORTER (Gene Therapy Systems Inc., San Diego, CA) (formulation of a neutral lipid (Dope) and a cationic lipid) and FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN) (Multi-component lipid based non-liposomal reagent).

Please replace the paragraphs beginning on page 44, line 3, through page 46, line 3, with the following paragraphs:

For preparation of purified genomic DNA, sorted chromosome samples were brought to 0.5% SDS, 50 mm EDTA and 100 μg/ml Proteinase K, then incubated for 18 hours at 50°C. 1 μl of a 20 mg/ml glycogen solution (Boehringer Mannheim) was added to each sample, followed by extraction with an equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1). After centrifugation at 21,000Xg for 10 min, the aqueous phases were transferred to fresh microfuge tubes and were re-extracted as above. 0.2 volumes of 10 M NH_4OAC, 1 μl of 20 mg/ml glycogen and 1 volume of iso-propanol were added to the twice extracted aqueous phases which were then vortexed and centrifuged for 15 minutes at 30,000Xg (at room temperature). Pellets were washed with 200 μl of 70% ethanol and re-centrifuged as above. The washed pellets were air-dried then resuspended in 5mM Tris-Cl, pH 8.0 at 0.5-2X10^6 chromosome equivalents/μl.

PCR was carried out on DNA prepared from sorted chromosome samples essentially as described (see, Co *et al.* (2000) *Chromosome Research 8*:183-191) using primers sets specific for EGFP and RAPSYN. Briefly, 50 μl PCR reactions were carried out on genomic DNA equivalent to 10,000 or 1000 chromosomes in a solution containing 10 mM Tris-Cl, pH 8.3, 50mM KCl, 200 μM dNTPs, 500 nM of forward and reverse primers, 1.5 mM $MgCl_2$, 1.25 units Taq polymerase (Ampli-Taq, Perkin-Elmer Cetus, CA). Separate reactions were carried out for each primer set. The reaction conditions were as follows: one cycle of 10 min. at 95°C, then 35 cycles of 1 min. at 94°C, 1 min. at 55°C, 1 min at 72°C, and finally one cycle of 10 min at 72°C. After completion the samples were held at 4°C until analyzed by agarose gel electrophoresis using the following primers (SEQ ID Nos. 1-4, respectively):

EGFP forward primer 5'-cgtccaggagcgcaccatcttctt-3';

EGFP reverse primer 3'-atcgcgcttctcgttggggtcttt-3';

RAPSYN forward primer 5'-aggactgggtggcttccaactcccagacac-3'; and

RAPSYN reverse primer 5'-agcttctcattgctgcgcgccaggttcagg-3'.

All primers were obtained from Canadian Life Technologies, Burlington, ON.

EXAMPLE 2

Preparation of Cationic vesicles

Vesicles were prepared at a lipid concentration of 700 nmol/ml lipid (cationic lipid/DOPE 1:1) as follows. In a glass tube (10ml) 350 nmol cationic lipid (SAINT-2) was mixed with 350nmol dioleoylphosphatidylethanolamine (DOPE), both solubilized in an organic solvent (Chloroform, Methanol or Chloroform/Methanol 1:1, v/v). Dioleoylphosphatidylethanolamine (DOPE; Avanti Polar Lipids, Alabaster, AL) forms inverse hexagonal phases in a membrane and weakens the membrane. Other effectors that may be used are *cis*-unsaturated phosphatidylethanolamines, *cis*-unsaturated fatty acids, and cholesterol. *Cis*-unsaturated phosphatidylcholines are less effective.

The solvent was evaporated under a stream of nitrogen (15 min/ 250 μl solvent at room temperature). The remaining solvent was removed totally by drying the lipid for 15 min in a desiccator under high vacuum from a vacuum pump. To the dried mixture was added l ml ultrapure water. This was vortexed vigorously for about 5 min. The resulting solution was sonicated in an ultrasonication bath (Laboratory Supplies Inc. NY) until a clear solution was obtained. The resulting suspension contained a population of unilamellar vesicles with a size distribution between 50 to 100 nm.

EXAMPLE 3

Preparation of Cationic vesicles via alcoholic injection

In a glass tube (10ml) 350 nmol cationic lipid (Saint-2) was mixed with 350 nmol DOPE, both solubilized in an organic solvent (chloroform, methanol or chloroform/methanol 1/1). The solvent was evaporated under a stream of nitrogen (15 min/ 250 μl solvent at room temperature). The remaining solvent was removed totally by drying the lipid for 15 min under high vacuum. This was then reconstituted in 100 μl pure ethanol.

Please replace the paragraphs beginning on page 50, line 8, through page 51, line 4, with the following paragraphs:

Transfection of the cells was performed as follows. The medium was removed from the cells, and the cells were washed twice with HBSS (Hanks balanced salt solution without Phenol Red (Gibco BRL, UK)) at 37°C. Then 500μl HBSS at 37°C was added per well, followed by 10 μl of the freshly prepared vesicle solution (prepared in Example 2) to yield a final concentration of 23.3 nmol/ml.

Alternatively, the medium was removed from the cells, and the cells were washed twice with HBSS. 500 μl HBSS/lipid solution at 37°C was added to each well. The HBSS/lipid solution was prepared by adding 1 μl ethanolic lipid solution (prepared as described above) to 500 μl HBSS under vigorous vortexing. The plates were then sealed with parafilm tape and shaken gently at room temperature for 30 min. After incubation, ultrasound was applied at an output energy of 0.5 Watt/cm^2 for 60 sec through the bottom of the plate to the cells. The ultrasound was mediated by an ultrasound gel (Aquasonic 100, Parker, NJ) between transducer and plate. The ultrasound was applied with an ImaRx Sonoporator 100. Immediately after applying ultrasound one GFP chromosome per seeded cell (2×10^5 - 5×10^5) (prepared in Example 1) was added. The plate was then sealed again and shaken gently for 1 h at room temperature. After the incubation 1ml medium (CHO-S-SFM 2 with 10% Fetal Calf Serum, 10000 μg/ml Penicillin and 10000 μg/ml Streptomycin Gibco BRL, Paisley, UK) was added to each well and the cells were incubated for 24 h at 37°C. The cells were then washed with medium, 1 ml medium was added, and the cells were incubated at 37° for another 24 h. Detection of expressed genes was then assayed by microscopy or detection of the transferred chromosome by FISH analysis. The negative control was performed in the same way, but with no chromosomes added to the cells.

Please replace the paragraph on page 51, lines 11-17, with the following paragraph:

B. Ultrasound mediated transfection of Hep-G2 cells with Saint-2

Hep-G2 cells were grown at 37°C, 5% CO_2, in DMEM with 4500 mg/l Glucose, with Pyridoxine/HCl, 10% Fetal Calf Serum, 10000 μg/ml Streptomycin and 1000 μg/ml Penicillin. Between 2 x 10^5 and 5 x 10^5 cells were plated onto sterile glass slides in a 12 wells plate 24 hours before usage.

Please replace the paragraph on page 51, lines 24-29, with the following paragraph:

C. Ultrasound mediated transfection of A9 cells with Saint-2

A9 cells were grown at 37°C, 5% CO_2, in DMEM with 4500 mg/l Glucose, with Pyridoxine/HCl, 10% Fetal Calf Serum, 10000 μg/ml Streptomycin and 10000 μg/ml Penicillin (GIBCO BRL, Paisley, UK). Between 2 x 10^5 and 5 x 10^5 cells were plated onto sterile glass slides in a 12 well plate 24 h before usage.

Please replace the paragraphs beginning on page 52, line 7, through page 54, line 6, with the following paragraphs:

EXAMPLE 7

A flow cytometry technique for measuring delivery of artificial chromosomes

Production cells lines (see Example 1) were grown in MEM medium (Gibco BRL) with 10% fetal calf serum (Can Sera, Rexdale ON) with 0.168 μg/ml hygromycin B (Calbiochem, San Diego, CA). Iododeoxyuridine or Bromodeoxyuridine was added directly to culture medium of the production cell line (CHO E42019) in the exponential phase of growth. Stock Iododeoxyuridine was made in tris base pH 10, Bromodeoxyuridine stocks in PBS. Final concentrations of 0.05-1 μM for continuous label of 20-24 hours of 5-50 μM with 15 minute pulse. After 24 hours, exponentially growing cells were blocked in mitosis with colchicine (1.0 μg/ml for 7 hours before harvest. Chromosomes

were then isolated and stained with Hoechst 33258 (2.5 μg/ml) and chromomycin A3 (50 μg/ml). Purification of artificial chromosomes was performed using a FACS Vantage flow cytometer (Becton Dickinson Immunocytometry systems, San Jose, CA). Chromomycin A3 was excited with the primary laser set at 457 nm, with emission detected using 475 nm long pass filter. Hoechst was excited by the secondary UV laser and emission detected using a 420/44 nm band-pass filter. Both lasers had an output of 150 mW. Bivariate distribution showing cell karyotype was accumulated from each sort. ACes were gated from other chromosomes and sorted. Condensing agents (hexylene glycol, spermine, and spermidine) were added to the sheath buffer to maintain condensed intact chromosome after sorting. IdU labeling index of sorted chromosomes was determined microscopically. Aliquot (2-10 μl) of sorted chromosomes was fixed in 0.2% formaldehyde solution for 5 minutes before being dried on clean microscopic slide. Microscope sample was fixed with 70% ethanol. Air-dried slide was denatured in coplin jar with 2N HCl for 30 minutes at room temperature and washed 2-3 times with PBS. Non specific binding was blocked with PBS and 4% BSA or serum for minimum of 10 minutes. A 1/5 dilution of FITC conjugated IdU/BrdU antibody (Becton Dickinson) with a final volume of 60-100 μl was applied to slide. Plastic strips, Durra seal (Diversified Biotech, Boston, MA) were overlaid on slides, and slides were kept in dark at 4%C in humidified covered box for 8-24 hours. DAPI (Sigma) 1 μg/ml in Vectorshield was used as counterstain. Fluorescence was detected using Zeiss axioplan 2 microscope equipped for epiflorescence. Minimum of 100 chromosomes was scored for determining % labeled. Unlabeled chromosomes were used as negative control.

The day before the transfection, trypsinize V79-4 (Chinese Hamster Lung fibroblast) cells and plate at 250,000 into a 6 well petri dish in 4 ml DMEM (Dulbecco's Modified Eagle Medium, Life Technologies) and 10% FBS (Can Sera Rexdale ON). The protocol was modified for use with LM (tk-) cell line by plating 500,000 cells. Lipid or dendrimer reagent was added to 1 X10^6 ACes

centrifugation. Add 20 μl of IdU/BrdU FITC conjugated B44 clone antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) to pellet and leave for 2 hours at room temperature in the dark with agitation every 30 minutes. Wash cells with block/permeabilization buffer and resuspend in PBS for flow analysis.

FLOW CYTOMETRY DETECTION OF FLUORESCENT IDUrd LABELED ACes

Percentage of transfected cells containing IdU labeled ACes was determined using a flow cytometry with an argon laser turned to 488 nm at 400 mW. FITC fluorescence was collected through a standard FITC 530/30-nm band pass filter. Cell populations were gated on the basis of side scatter versus forward scatter to exclude debris and doublets. Data were accumulated (15,000 events) to form bivariate channel distribution showing forward scatter versus green fluorescence (IdU-FITC). The fluorescence level at which cells were determined to be positive was established by visual inspection of the histogram of negative control cells, such that approximately 1% appeared in the positive region.

Results:

The transfection delivery results of IdU labeled ACes are set forth in Table 2.

IN THE CLAIMS:

Please replace claims 9, 10, 11, 13, 15, 16, 20, 21, and 27 with the following claims (a marked-up copy of the amended specification is attached to this Amendment):

9. (Amended) The method of claim 23, wherein step (a) comprises contacting the nucleic acid molecule with a delivery agent that comprises a cationic compound.

10. (Amended) The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-

dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

11. (Amended) The method of claim 1, wherein the nucleic acid molecules are natural chromosomes, artificial chromosomes, fragments of a chromosome or naked DNA that is greater than at least about 0.6 megabase in size.

13. (Amended) The method of claim 1, wherein the nucleic acid molecules are artificial chromosome expression systems (ACes).

15. (Amended) The method of claim 14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. (Amended) The method of claim 14, wherein the cells, are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

20. (Amended) The method of claim 17, wherein a delivery agent comprises a cationic compound, and the nucleic acid molecule is treated therewith.

21. (Amended) The method of claim 20, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

27. (Amended) The method of claim 26, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P)$, $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

REMARKS

Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 50-1213.

The specification is amended to correct typographical and spelling errors and to produce grammatical clarity. The specification is also amended to delete the inadvertently typed letter "d" and to add the inadvertently omitted word "cytometry". The basis for this amendment is found in the specification, in particular page 6, line 23, which describes flow cytometry as a detection method. The specification is also amended to add the inadvertently omitted phrase "of a nucleic acid molecule can occur" for grammatical clarity. The basis for this amendment is found in the specification, in particular page 8, lines 11-12, which describes delivery of a nucleic acid molecule. The specification is also amended to correct the names of chemical compounds. The bases for these amendments are found in the specification, in particular page 7, line 5, for iododeoxyuridine and bromodeoxyuridine and page 20, lines 9-13, for dioleoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, phosphatidylethanolamine, and phosphatidylcholine. The specification is also amended to delete the second occurrence of the word

"dipalmitoylphosphatidylethanolamine" on page 20, line 11, to remove redundancy and produce grammatical clarity.

The amendments to claims 13 and 15 correct tyopographical errors and produce grammatical clarity. The amendment to claim 9 corrects claim dependency and adds the inadvertently omitted word "wherein" for grammatical clarity. The amendments to claims 11, 16, and 20 correct claim dependency. The amendments to claims 10, 21, and 27 delete the second occurrence of the word "dioleoylphosphatidylethanolamine (DOPE)" to remove redundancy and produce grammatical clarity. No new matter has been added.

Included as an attachment is a marked-up version of the specification paragraphs that are being amended, per 37 CFR §1.121.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________________
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: De Jong *et al.*

Serial No.: 09/815,981

Filed: March 22, 2001

For: *METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF*

Art Unit: Unassigned

Examiner: Unassigned

ATTACHMENT TO THE PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph on page 5, lines 3-7, as follows:

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be one that encodes fluorescent protein, enzyme, such as a luciferase, or antibody. The delivered nucleic acid molecules include, but are not limited to, RNA, including ribozymes, DNA, including naked DNA and chromosomes, plasmids, chromosome fragments, typically containing at least one gene or at least 1 Kb, naked DNA, or natural chromosomes. The method is exemplified herein by determining delivery and expression of artificial chromosome expression systems ([Aces]ACes). Any types of cells, eukaryotic and prokaryotic, including cell lines, primary cell lines, plant cells, and animal cells, including stem cells, embryonic cells, and other cells into which delivery of a nucleic acid molecule can occur is contemplated.

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Please amend the paragraph beginning on page 15, line 18, through page 16, line 4, as follows:

As used herein, gene therapy involves the transfer or insertion of nucleic acid molecules, and, in particular, large nucleic acid molecules, into certain cells,

which are also referred to as target cells, to produce specific gene products that are involved in correcting or modulating diseases or disorders. The nucleic acid is introduced into the selected target cells in a manner such that the nucleic acid is expressed and a product encoded thereby is produced. Alternatively, the nucleic acid may in some manner mediate expression of DNA that encodes a therapeutic product. This product may be a therapeutic compound, which is produced in therapeutically effective amounts or at a therapeutically useful time. It may also encode a product, such as a peptide or RNA, that in some manner mediates, directly or indirectly, expression of a therapeutic product. Expression of the nucleic acid by the target cells within an organism afflicted with a disease or disorder thereby [providing] <u>provides</u> a way to modulate the disease or disorder. The nucleic acid encoding the therapeutic product may be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

Please amend the paragraph beginning on page 16, line 24, through page 17, line 8, as follows:

As used herein, a reporter gene construct is a DNA molecule that includes a reporter gene operatively linked to a transcriptional control sequence. The transcriptional control sequences include the promoter and other optional regulatory regions, such as enhancer sequences, that modulate the activity of the promoter, or control sequences that modulate the activity or efficiency of the RNA polymerase that recognizes the promoter, or control sequences <u>that</u> are recognized by effector molecules, including those that are specifically induced by interaction of an extracellular signal with a cell surface protein. For example, modulation of the activity of the promoter may be effected by altering the RNA polymerase binding to the promoter region, or, alternatively, by interfering with initiation of transcription or elongation of the mRNA. Such sequences are herein collectively referred to as transcriptional control elements or sequences. In addition, the construct may include sequences of nucleotides that alter

translation of the resulting mRNA, thereby altering the amount of reporter gene product.

Please amend the paragraphs beginning on page 19, line 24, through page 20, line 18, as follows:

Cationic Compounds

Cationic compounds for use in the methods provided herein are available commercially or can be synthesized by those of skill in the art. Any cationic compound may <u>be</u> used for delivery of nucleic acid molecules, such as DNA, into a particular cell type using the provided methods. One of skill in the art by using the provided screening procedures can readily determine which of the cationic compounds are best suited for delivery of specific nucleic acid molecules, such as DNA, into a specific target cell type.

(a) Cationic Lipids

Cationic lipid reagents can be classified into two general categories based on the number of positive charges in the lipid headgroup; either a single positive charge or multiple positive charges, usually up to 5. Cationic lipids are often mixed with neutral lipids prior to use as delivery agents. Neutral lipids include, but are not limited to, lecithins; [phosphotidylethanolamine] <u>phosphatidylethanolamine</u>; phosphatidylethanolamines, such as DOPE (dioleoylphosphatidylethanolamine), DPPE (dipalmitoylphosphatidyl-ethanolamine), [dipalmiteoylphosphatidylethanolamine,] POPE (palmitoyloleoyl-phosphatidylethanolamine) and distearoylphosphatidylethanolamine; [phosphotidylcholine] <u>phosphatidylcholine</u>; phosphatidylcholines, such as DOPC ([dioleoylphosphidylcholine] <u>dioleoylphosphatidylcholine</u>), DPPC (dipalmitoyl-phosphatidylcholine)<u>,</u> POPC (palmitoyloleoylphosphatidylcholine) and distearoyl-phosphatidylcholine; fatty acid esters; glycerol esters; sphingolipids; cardiolipin; cerebrosides; and ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3βOH-sterols.

Please amend the paragraph beginning on page 20, line 24, through page 25, line 18, as follows:

Examples of cationic lipid compounds include, but are not limited to: Lipofectin (Life Technologies, Inc., Burlington, Ont.)(1:1 (w/w) formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dioleoylphosphatidylethanolamine (DOPE)[)]; LipofectAMINE (Life Technologies, Burlington, Ont., see U.S. Patent No. 5,334,761) (3:1 (w/w) formulation of polycationic lipid 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) and [dioleoyl phosphatidylethanolamine] dioleoylphosphatidylethanolamine (DOPE)[)], LipofectAMINE PLUS (Life Technologies, Burlington, Ont. see U.S. Patent Nos. 5,334,761 and 5,736,392; see, also U.S. Patent No. 6,051,429) (LipofectAmine and Plus reagent), LipofectAMINE 2000 (Life Technologies, Burlington, Ont.; see also International PCT application No. WO 00/27795) (Cationic lipid), Effectene (Qiagen, Inc., Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, Inc., San Luis Obispo, CA) (ethanolic cationic lipids numbers 1 through 12: $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$); Cytofectene (Bio-Rad, Hercules, CA) (mixture of a cationic lipid and a neutral lipid), GenePORTER (Gene Therapy Systems Inc., San Diego, CA) (formulation of a neutral lipid (Dope) and a cationic lipid) and FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN) (Multi-component lipid based non-liposomal reagent).

Please amend the paragraphs beginning on page 44, line 3, through page 46, line 3, as follows:

For preparation of purified genomic DNA, sorted chromosome samples were brought to 0.5% SDS, 50 mm EDTA and 100 μg/ml Proteinase K, then incubated for 18 hours at 50°C. 1 μl of a 20 mg/ml glycogen solution (Boehringer Mannheim) was added to each sample, followed by extraction with an equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1). After centrifugation at 21,000Xg for 10 min, the aqueous phases were transferred to fresh microfuge tubes and were re-extracted as above. 0.2 volumes of 10 M NH_4OAC, 1 μl of 20 mg/ml glycogen and 1 volume of iso-propanol were added to the twice extracted aqueous phases which were then vortexed and centrifuged for 15 minutes at 30,000Xg (at room [temperaautre]<u>temperature</u>). Pellets were washed with 200 μl of 70% ethanol and re-centrifuged as above. The washed pellets were air-dried then resuspended in 5mM Tris-Cl, pH 8.0 at 0.5-2X10^6 chromosome equivalents/μl.

PCR was carried out on DNA prepared from sorted chromosome samples essentially as described (see, Co *et al.* (2000) *Chromosome Research 8*:183-191) using primers sets specific for EGFP and RAPSYN. Briefly, 50 μl PCR reactions were carried out on genomic DNA equivalent to 10,000 or 1000 chromosomes in a solution containing 10 mM Tris-Cl, pH 8.3, 50mM KCl, 200 μM dNTPs, 500 nM of forward and reverse primers, 1.5 mM $MgCl_2$, 1.25 units Taq polymerase (Ampli-Taq, Perkin-Elmer Cetus, CA). Separate reactions were carried out for each primer set. The reaction conditions were as follows: one cycle of 10 min. at 95°C, then 35 cycles of 1 min. <u>at</u> 94°C, 1 min. <u>at</u> 55°C, 1 min <u>at</u> 72°C, and finally one cycle of 10 min at 72°C. After completion the samples were held at 4°C until analyzed by agarose gel electrophoresis using the following primers (SEQ ID Nos. 1-4, respectively):
EGFP forward primer 5'-cgtccaggagcgcaccatcttctt-3';
EGFP reverse primer 3'-atcgcgcttctcgttggggtcttt-3';
RAPSYN forward primer 5'-aggactgggtggcttccaactcccagacac-3'; and

RAPSYN reverse primer 5'-agcttctcattgctgcgcgccaggttcagg-3'.

All primers were obtained from Canadian Life Technologies, Burlington, ON.

EXAMPLE 2

Preparation of Cationic vesicles

Vesicles were prepared at a lipid concentration of 700 [nmoles]nmol/ml lipid (cationic lipid/DOPE 1:1) as follows. In a glass tube (10ml) 350 [nmoles]nmol cationic lipid (SAINT-2) was mixed with 350[nmoles]nmol [dioleylphosphoethanolamine] dioleoylphosphatidylethanolamine (DOPE), both solubilized in an organic solvent (Chloroform, Methanol or Chloroform/Methanol 1:1, v/v). [Diphosphatidylethanolamine] Dioleoylphosphatidylethanolamine (DOPE; Avanti Polar Lipids, Alabaster, AL) forms inverse hexagonal phases in a membrane and weakens the membrane. Other effectors that may be used are *cis*-unsaturated [phosphoethanolamines] phosphatidylethanolamines, *cis*-unsaturated fatty acids, and cholesterol. *Cis*-unsaturated phosphatidylcholines are less effective.

The solvent was evaporated under a stream of nitrogen (15 min/ 250 μl solvent at room temperature). The remaining solvent was removed totally by drying the lipid for 15 min in [an] a desiccator under high vacuum from a vacuum pump. To the dried mixture was added l ml ultrapure water. This was vortexed vigorously for about 5 min. The resulting solution was sonicated in an ultrasonication bath (Laboratory Supplies Inc. NY) until a clear solution was obtained. The resulting suspension contained a population of unilamellar vesicles with a size distribution between 50 to 100 nm.

EXAMPLE 3

Preparation of Cationic vesicles via alcoholic injection

In a glass tube (10ml) 350 [nmoles]nmol cationic lipid (Saint-2) was mixed with 350 [nmoles]nmol DOPE, both solubilized in an organic solvent (chloroform, methanol or chloroform/methanol 1/1). The solvent was evaporated under a stream of nitrogen (15 min/ 250 μl solvent at room temperature). The

remaining solvent was removed totally by drying the lipid for 15 min under high vacuum. This was then reconstituted in 100 μl pure ethanol.

Please amend the paragraphs beginning on page 50, line 8, through page 51, line 4, as follows:

Transfection of the cells was performed as follows. The medium was removed from the cells, and the cells were washed twice with HBSS (Hanks balanced salt solution without Phenol Red (Gibco BRL, UK)) at 37°C. Then 500μl HBSS at 37°C was added per well, followed by 10 μl of the freshly prepared vesicle solution (prepared in Example 2) to yield a final concentration of 23.3 [nmole]<u>nmol</u>/ml.

Alternatively, the medium was removed from the cells, and the cells were washed twice with HBSS. 500 μl HBSS/lipid solution at 37°C was added to each well. The HBSS/lipid solution was prepared by adding 1 μl ethanolic lipid solution (prepared as described above) to 500 μl HBSS under vigorous vortexing. The plates were then sealed with parafilm tape and shaken gently at room temperature for 30 min. After incubation, ultrasound was applied at an output energy of 0.5 Watt/cm^2 for 60 sec through the bottom of the plate to the cells. The ultrasound was mediated by an ultrasound gel (Aquasonic 100, Parker, NJ) between transducer and plate. The ultrasound was applied with an ImaRx Sonoporator 100. Immediately after applying ultrasound one GFP chromosome per seeded cell (2×10^5 - 5×10^5) (prepared in Example 1) was added. The plate was then sealed again and shaken gently for 1 h at room temperature. After the incubation 1ml medium (CHO-S-SFM 2 with 10% Fetal Calf Serum, 10000 μg/ml Penicillin and 10000 μg/ml Streptomycin Gibco BRL, Paisley, UK) was added to each well and the cells were incubated for 24 h at 37°C. The cells were then washed with medium<u>,</u>[and] 1 ml medium was added<u>,</u> and the cells were incubated at 37° for another 24 h. Detection of expressed genes was then assayed by microscopy or detection of the transferred chromosome by FISH analysis.

The negative control was performed in the same way, but with no chromosomes added to the cells.

Please amend the paragraph on page 51, lines 11-17, as follows:

B. Ultrasound mediated transfection of Hep-G2 cells with Saint-2

Hep-G2 cells were grown at 37°C, 5% CO_2, in DMEM with 4500 mg/l Glucose, with Pyridoxine/[HCL]<u>HCl</u>, 10% Fetal Calf Serum, 10000 μg/ml Streptomycin and 1000 μg/ml Penicillin. Between 2 x 10^5 and 5 x 10^5 cells were plated onto sterile glass slides in a 12 wells plate 24 hours before usage.

Please amend the paragraph on page 51, lines 24-29, as follows:

C. Ultrasound mediated transfection of A9 cells with Saint-2

A9 cells were grown at 37°C, 5% CO_2, in DMEM with 4500 mg/l Glucose, with Pyridoxine/[HCL]<u>HCl</u>, 10% Fetal Calf Serum, 10000 μg/ml Streptomycin and 10000 μg/ml Penicillin (GIBCO BRL, Paisley, UK). Between 2 x 10^5 and 5 x 10^5 cells were plated onto sterile glass slides in a 12 well plate 24 h before usage.

Please amend the paragraphs beginning on page 52, line 7, through page 54, line 6, as follows:

EXAMPLE 7

A flow cytometry technique for measuring delivery of artificial chromosomes

Production cells lines (see Example 1) were grown in MEM medium (Gibco BRL) with 10% fetal calf serum (Can Sera, Rexdale ON) with 0.168 μg/ml hygromycin B (Calbiochem, San Diego, CA). [Iododeoxyurine] <u>Iododeoxyuridine</u> or [Bromodeoxyurdine] <u>Bromodeoxyuridine</u> [were] <u>was</u> added directly to culture medium of the production cell line (CHO E42019) in the exponential phase of growth. Stock [Iododeoxyurdine] <u>Iododeoxyuridine</u> <u>was</u> made in tris base pH 10[.]<u>,</u> [Bromodeoxyurdine] <u>Bromodeoxyuridine</u> stocks in PBS. Final concentrations of 0.05-1 μM for continuous label of 20-24 hours of 5-50 μM

with 15 minute pulse. After 24 hours, exponentially growing cells <u>were</u> blocked in mitosis with colchicine (1.0 μg/ml for 7 hours before harvest. Chromosomes were then isolated and stained with Hoechst 33258 (2.5 μg/ml) and chromomycin A3 (50 μg/ml). Purification of artificial chromosomes was performed using a FACS Vantage flow cytometer (Becton Dickinson Immunocytometry systems, San Jose, CA). Chromomycin A3 was excited with the primary laser set at 457 nm, with emission detected using 475 nm long pass filter. Hoechst was excited by the secondary UV laser and emission detected using a 420/44 nm band-pass filter. Both lasers had an output of 150 mW. Bivariate distribution showing cell karyotype was accumulated from each sort. ACes were gated from other chromosomes and sorted. Condensing agents (hexylene glycol, spermine, and spermidine) were added to the sheath buffer to maintain condensed intact chromosome after sorting. IdU labeling index of sorted chromosomes was determined microscopically. Aliquot (2-10 μl) of sorted chromosomes <u>was</u> fixed in 0.2% formaldehyde solution for 5 minutes before being dried on clean microscopic slide. Microscope sample <u>was</u> fixed with 70% ethanol. Air-dried slide <u>was</u> denatured in coplin jar with [2NHCL]<u>2N HCl</u> for 30 minutes at room temperature and washed 2-3 times with PBS. Non specific binding <u>was</u> blocked with PBS and 4% BSA or serum for minimum of 10 minutes. A 1/5 dilution of FITC conjugated IdU/BrdU antibody (Becton Dickinson) with a final volume of 60-100 μl <u>was</u> applied to slide. Plastic strips, Durra seal (Diversified Biotech, Boston, MA) <u>were</u> overlaid on slides, and slides <u>were</u> kept in dark at 4%C in humidified covered box for 8-24 hours. DAPI (Sigma) 1 μg/ml in Vectorshield <u>was</u> used as counterstain. Fluorescence <u>was</u> detected using Zeiss axioplan 2 microscope equipped for epiflorescence. Minimum of 100 chromosomes <u>was</u> scored for determining % labeled. Unlabeled chromosomes <u>were</u> used as negative control.

The day before the transfection, trypsinize V79-4 (Chinese Hamster Lung fibroblast) cells and plate at 250,000 into a 6 well petri dish in 4 [mls]<u>ml</u> DMEM (Dulbecco's Modified Eagle Medium, Life Technologies) and 10% FBS (Can Sera

Rexdale ON). The protocol was modified for use with LM (tk-) cell line by plating 500,000 cells. Lipid or dendrimer reagent was added to 1 X10^6 ACes sorted in ~800 μl sort buffer. Exemplary protocol variations are set forth in Table 1. Chromosome and transfection agents were mixed gently. Complexes were added to cells drop-wise and plate swirled to mix. Plates were kept at 37°C in a 5% CO_2 incubator for specified transfection time. The volume in a well was then made up to 4-5 ml with DMEM and 10% FBS. Recipient cells left for 24 hours at 37°C in a 5% CO_2 incubator. Trypsinize transfected cells. Samples to be analyzed for IdU labeled chromosome delivery are fixed in cold 70% ethanol and stored at -20°C, [tp]to be ready for IdU antibody staining. Samples to be grown for colony selection are counted and then transferred to 10-cm dishes at densities of 10,000 and 100,000 cells in duplicate with remaining cells put in a 15 cm dish. After 24 hours, selection medium containing of DMEM and 10% FBS with 0.7 mg/ml hygromycin B, # 400051 (Calbiochem San Diego, CA) is added. Selection medium is changed every 2-3 days. This concentration of hygromycin B kills the wild type cells after selection for 7 days. At 10-14 days colonies are expanded and then screened by FISH for intact chromosome transfer and assayed for beta galactosidase expression.

Please amend the paragraphs beginning on page 55, line 7, through page 56, line 8, as follows:

IDU ANTIBODY LABELING

A standard BrdU staining flow cytometry protocol (Gratzer et al. Cytometry (1981);6:385-393) is used except with some modifications at neutralization step, the presence of detergent during denaturation and the composition of blocking buffer. Between each step samples are centrifuged at 300 g for 7-10 minutes and supernatant removed. Samples of 1-2 million cells are fixed in 70% cold ethanol. Cells are then denatured in 1-2 ml of 2N [HCL]HCl plus 0.5% triton X for 30 minutes at room temperature. Sample undergoes 3-4 washes with cold DMEM until indictor is neutral. Final wash

with cold DMEM plus 5% FBS. Blocking/permeabilization buffer containing PBS, 0.1% triton X and 4% FBS is added for 10-15 minutes before pelleting sample by centrifugation. Add 20 μl of IdU/BrdU FITC conjugated B44 clone antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) to pellet and leave for 2 hours at room temperature in the dark with agitation every 30 minutes. Wash cells with block/permeabilization buffer and resuspend in PBS for flow analysis.

FLOW CYTOMETRY DETECTION OF FLUORESCENT [IDUrdD]IDUrd LABELED ACes

Percentage of transfected cells containing IdU labeled ACes was determined using a flow cytometry with an argon laser turned to 488 nm at 400 mW. FITC fluorescence was collected through a standard FITC 530/30-nm band pass filter. Cell populations were gated on the basis of side scatter versus forward scatter to exclude debris and doublets. Data [was]were accumulated (15,000 events) to form bivariate channel distribution showing forward scatter versus green fluorescence (IdU-FITC). The fluorescence level at which cells were determined to be positive was established by visual inspection of the histogram of negative control cells, such that approximately 1% appeared in the positive region.

Results:

The transfection delivery results of IdU labeled ACes are set forth in Table 2.

IN THE CLAIMS:

Please amend the claims 9, 10, 11, 13, 15, 16, 20, 21, and 27 as follows:

9. (Amended) The method of claim [16] 23, wherein step (a) comprises contacting the nucleic acid molecule [is] with a delivery agent that comprises a cationic compound.

10. (Amended) The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), [dioleoyl phosphatidylethanolamine (DOPE),] $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

11. (Amended) The method of claim [10] 1, wherein the nucleic acid molecules are natural chromosomes, artificial chromosomes, fragments of a chromosome or naked DNA that is greater than at least about 0.6 megabase in size.

13. (Amended) The method of claim 1, wherein the nucleic acid molecules are artificial chromosome expression systems ([Aces]ACes).

15. (Amended) The method of claim 14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. (Amended) The method of claim [15] 14, wherein the cells, are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

20. (Amended) The method of claim [19] 17, wherein a delivery agent comprises a cationic compound, and the nucleic acid molecule is treated therewith.

21. (Amended) The method of claim 20, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), [dioleoyl phosphatidylethanolamine (DOPE),] $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$,

$C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

27. (Amended) The method of claim 26, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), [dioleoyl phosphatidylethanolamine (DOPE),] $C_{52}H_{106}N_6O_4{\cdot}4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: de Jong *et al.*
Serial No.: 09/815,981
Conf. No.: 7622
Cust No.: 24961
Filed: March 22, 2001
For: ***METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF***
Art Unit: 1636
Examiner: Sullivan, D.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Date of Deposit June 16, 2003

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06/16/03 Debra Walker
Date Debra Walker

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Transmitted herewith are an Amendment and Response responsive to the Office Action mailed December 16, 2002, a check for the requisite fee for a three month extension of time ($465), and a return postcard for filing in connection with the above captioned application. If a Petition for extension of time is required, this paper is to be considered such Petition.

☒ The Commissioner is hereby authorized to charge any fee, including any submitted herewith if the attached check(s) is in the wrong amount or otherwise improper or missing, that may be due in connection with this and the attached papers, or with this application during its entire pendency to or to credit any overpayment to Deposit Account No. 50-1213. A duplicate of this sheet is enclosed.

Respectfully submitted,
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> nucleic acid molecules typically may be on the order of about 10 to about 450 or more Mbase, and may be of various sizes, such as, for example, from about 250 to about 400 Mbase, about 150 to about 200 Mbase, about 90 to about 120 Mbase, about 60 to about 100 Mbase and about 15 to 50 Mbase.
>
> Examples of large nucleic acid molecules include, but are not limited to, natural chromosomes and fragments thereof, especially mammalian chromosomes and fragments thereof which retain a centromere and telomeres, artificial chromosome expression systems (ACes; also called satellite DNA-based artificial chromosomes (SATACs); see U.S. Patent Nos. 6,025,155 and 6,077,697), mammalian artificial chromosomes (MACs), plant artificial chromosomes, insect artificial chromosomes, avian artificial chromosomes and minichromosomes (see, *e.g.*, 5,712,134, 5,891,691 and 5,288,625).

Claim 9 is amended to more particularly point out the claimed subject matter. Claim 9 is amended to include the phrase "introducing the labelled large nucleic acid molecules" to provide reflect the antecedent basis provided in Claims 1 and 6, from which Claim 9 depends. Basis for "introducing labelled large nucleic acid molecules" with a cationic delivery agent as recited in Claim 9 also is found in the specification, *e.g.*, at page 20, line 4 through page 22, line 4 and also in the working examples, Table 1, pages 54-55.

The amendment of the specification corrects minor typographical errors. No new matter is added.

An executed DECLARATION under 37 C.F.R. §1.132 of Vanderbyl, with reference publication, is attached hereto, as is a marked up copy per 37 C.F.R. §1.121 of the amended paragraph and claims. A Supplemental Information Disclosure Statement also is filed on the same day herewith under separate cover.

OBJECTION TO CLAIMS 1, 3 AND 30

Claims 1, 3 and 30 are objected to because of informalities within the claims. This objection has been rendered moot by amending the claims to correct the minor typographical errors pointed to by the Examiner.

PROVISIONAL REJECTION OF CLAIMS 1-8, 11-16 AND 30 UNDER STATUTORY TYPE (35 U.S.C. § 101) DOUBLE PATENTING

Claims 1-8, 11-16 and 30 are provisionally rejected under statutory type (35 U.S.C. § 101) double patenting as allegedly being unpatentable over claims 1-8, 11-16 and 30 of copending Application No. 10/086,745. Specifically, it is alleged that claims 1-8, 11-16 and 30 of copending Application No. 10/086,745 are identical in scope to the claims of the instant application.

In the Election and Preliminary Amendment filed responsive to the Requirement for Restriction that issued September 19, 2002, in connection with the above-captioned application, Applicant stated that Claims 17-29, 31 and 32, which were cancelled as being drawn to non-elected subject matter, would be prosecuted in the aforementioned co-pending U.S. application Serial No. 10/086,745. Upon receipt of an Action in co-pending U.S. application Serial No. 10/086,745 on the merits, claims of identical scope to any pending in this case will be cancelled, thereby obviating any statutory double patenting issues, if any, between the pending claims of the two applications.

THE REJECTION OF CLAIMS 1-16 and 30 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1-16 and 30 are rejected under 35 U.S.C. § 112, first paragraph because it is alleged that the specification does not describe the subject matter in such a way as to enable one skilled in the art to obtain gene expression from a labelled nucleic acid molecule introduced into a cell. To support this allegation, the Office Action cites Felgner *et al.* (WO 99/13719), which allegedly demonstrates that standard methods of labeling DNA do not permit detection of structurally and functionally intact plasmid. Additionally, the Office Action cites Neves *et al.* (*Bioconjugate Chem* (2000) *11:51-55*), which allegedly demonstrates that labeling DNA with either tetraflurobenzylamido-lissamine or rhodamine-labelled nucleotides reduced or abolished reporter gene expression, respectively, and furthermore, cites Zelphati *et al.* (*Hum. Gene*

Therap (1999) *10:15-24*), which allegedly provides that labeling methods reduce or destroy the ability of DNA to be transcribed.

It is further alleged that while the working examples provide a method of introducing labelled ACes carrying a reporter gene and Applicant states that reporter gene expression was measured, no evidence of detection of reporter gene expression from labelled ACes is provided. The Examiner urges that the statement by Applicant at page 35 of the specification, indicating that labelling of ACes does not affect gene expression, is not supported by data. The Examiner concludes that the lack of supporting data in the specification, when combined with the teachings in the art indicating that incorporation of labeled nucleotide analogs adversely affects gene expression, shows that the degree of experimentation by the skilled artisan to achieve expression from labelled nucleic acid molecules is undue.

This rejection is respectfully traversed. Although not needed, a DECLARATION under 37 C.F.R. §1.132 of Vanderbyl demonstrating such results is provided.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocchi et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

Furthermore, statements and disclosure in an application are presumed true:

> [a]s a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and describing the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.
>
> . . . it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure (In re Marzocchi & Horton, 58 CCPA 1069, 439 F. 2d 220, 169 USPQ 367, 369-370 (1971)).

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

1. Application of the Above-noted Factors

Scope of the claims

Claims 1-16 and 30 are directed to methods for detecting or determining nucleic acid delivery and expression in a cell by introducing labelled large nucleic acid molecules, detecting labelled cells and measuring reporter gene product to measure the delivery and expression, respectively. The dependent claims further specify labeling the DNA using iododeoxyuridine or bromodeoxyuridine and the use of flow cytometry, fluorometry, cell imaging or fluorescence spectroscopy for detection of labelled DNA. The dependent claims also specify types of reporter genes including fluorescent reporter genes, such as green, red and blue fluorescent proteins, and enzymes such as β-galactosidase, luciferase and alkaline phosphatase. The dependent claims further specify types of delivery agents and specific cell types to which the DNAs are delivered. Additionally, the dependent claims specify that the delivered nucleic acid may be natural chromosomes, artificial chromosomes, chromosome fragments, or naked DNA greater than about 0.6 megabases in size.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Teachings of the specification

The specification describes the delivery of DNA, including labelled DNA into cells and the expression of gene products encoded therein. The teachings of the specification describe how to: introduce labelled large nucleic acid to a cell; measure the delivery of the nucleic acid to the cell by detecting labelled cells; and determine the expression of the product of the reporter gene encoded by the nucleic acid.

Each of these steps are described in detail in the specification. The steps of the methods claimed herein are described in the specification in detail and the specification further provides working examples of particular embodiments thereof, including the steps of: introducing labelled nucleic acid into a cell; detecting the labelled nucleic acid as an indication of delivery; and measuring reporter gene product as an indication of gene expression. The specification also describes methods of detecting labelled nucleic acids, the use of various reporter genes, the use of different cell types, the use of different delivery agents, including various cationic lipids and the use of large nucleic acid molecules such as artificial chromosomes.

In addition, the specification exemplifies the steps of the method in numerous working examples. For example, the specification discloses methods for labeling large nucleic acid molecules with iododeoxyuridine and bromodeoxyuridine; methods of delivering labelled large nucleic acids to a variety of cell types using delivery agents such as cationic lipids, ultrasound and electroporation; methods of detecting nucleic acid delivery into the cell including

flow cytometry; and methods of detecting gene expression of reporter genes such as GFP and β-galactosidase encoded by the labelled DNA.

A) Methods for Labeling Nucleic Acids

The specification teaches methods for preparing labelled nucleic acids. Labeling includes the use of directly detectable labels, such as labeling with radioactivity (page 33, line 10) , or labels that are detectable upon binding to a cognate receptor (*e.g.*, antigen-antibody complex formation; page 33, lines 16-20). To illustrate the methods, the specification describes in extensive detail the the preparation of large nucleic acid molecules and the generation of cell lines with large nucleic acid molecules such as ACes (Example 1, section B, pages 40-41), labeling of DNA by the incorporation of iododeoxyuridine and bromodeoxyuridine (Example 7, page 52) and the purification of ACes (Example 1, section C, pages 43-45) and labelled ACes (Example 7, pages 52-53).

The specification further teaches methods for the delivery of labelled nucleic acids. These methods include the use of delivery agents such as cationic compounds (page 19, line 24 through page 22, line 4), the use of ultrasound (page 22, line 9 through page 23, line 19) and the use of electroporation (page 23, lines 20-26). The specification describes *in vitro*, *ex vivo* and *in vivo* delivery methods using these agents (page 23, line 27 through page 28, line 9). Additionally, these methods are exemplified in the Examples. Example 4 provides detailed procedures for transfection (page 46, line 5 through page 47, line 20) and transfection of labelled DNA is exemplified (page 47, lines 21-25, using the described methods). Furthermore, in Example 7, artificial chromosomes are labelled with either iododeoxyuridine (IdU) or bromodeoxyuridine (BrdU) (page 52, line 10 through page 53, line 14) and then transfected by a variety of methods (page 53, line 15 through page 55, line 5).

B) Methods for Measuring Labelled Nucleic Acid Delivery

The specification teaches methods for measuring the delivery of nucleic acid into cells by detecting labelled cells. The methods include using

microscopy and flow cytometry for detection of labelled cells (page 28, line 10 through page 29, line 12). Various parameters such as transfer efficiency, clonogenicity, viable fraction, potential transfection efficiency and chromos index for assessing the efficiency of delivery also are provided in detail (page 29, line 13 through page 33, line 2). Additionally, these methods are exemplified in Example 4 where cells transfected with IdU labelled ACes are assessed for the percentage of transfected cells using flow cytometry (page 47, line 21 through page 48, line 4). Additionally, Example 7 describes the measurement of DNA delivery (page 55, line 7 through page 56, line 5) and then details the results of such measurements in Table 2 of Example 7 (pages 56-57).

<u>C. Methods for Measuring Gene Expression from Labelled or Non-Labelled Nucleic Acids Delivered to Cells</u>

The specification teaches that reporter gene expression can be measured to assess expression of gene(s) encoded by the labelled large nucleic acid molecules that are delivered to cells. The specification describes numerous reporters that may be used including GFP, β-galactosidase, CAT and others (page 34, lines 21-28). The specification further teaches that reporter gene expression may be measured from labelled nucleic acid molecules (page 34, line 29 through page 35, line 15) and that labeling the nucleic acid molecules does not interfere with reporter gene expression (page 35, lines 9-15). Additionally, measurement of reporter genes is exemplified in several of the examples, including Example 1 (measurement of GFP; page 41, line 21 through page 42, line 23), Example 4 (measurement of β-galactosidase; page 47, lines 18-20), Examples 5 and 6 (GFP measurements; page 49, lines 26-31 and page 51, lines 6-8, respectively), and Example 7 (measurement of β-galactosidase; page 54, lines 4-6). Examples 4 and 7 include labelled ACes in the methods exemplified.

The Examiner alleges that while the specification states that "incorporation of the analog label does not affect GFP protein expression," no data is provided to support this assertion, which is allegedly "at odds with the teachings of the prior art." The Examiner asserts that it is therefore unclear as to whether this is a statement of fact or a prophetic statement.

It is respectfully submitted that the filing of a patent application serves as conception and constructive reduction to practice of the subject matter described and claimed in the application ((MPEP §2138.05; *Hyatt v. Boone*, 146 F.3d 1348, 1352, 47 USPQ2d 1128, 1130 (Fed. Cir. 1998)). Further, no working examples or supportive data need be provided in the application. Rather, the requirements of § 112, first paragraph can be fulfilled by broad terminology. *In re Marzocchi et al.*, 469 USPQ 367 (CCPA 1971). As stated above, statements and disclosure in an application are presumed true:

> [a]s a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and describing the subject matter sought to be patented <u>must</u> be taken as in compliance with the enabling requirement of the first paragraph of § 112 <u>unless</u> there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.
>
> . . . it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain <u>why</u> it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure (*In re Marzocchi & Horton*, 58 CCPA 1069, 439 F. 2d 220, 169 USPQ 367, 369-370 (1971)).

Furthermore, as discussed below, the "teachings of the prior art" alleged by the Examiner to be "at odds" with the instantly claimed subject matter bear little relevance to enablement of the steps of the instant methods as of their

filing date, and the Examiner has provided no basis to doubt the veracity of what is described and claimed.

As discussed above, Applicant is not required to provide data or illustrative examples in support of every assertion in the specification or everything within the scope of a broad claim (*In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973)). Nonetheless, as discussed below, the accompanying DECLARATION under 37 C.F.R. § 1.132 of Sandra Vanderbyl demonstrates that by following the steps of the instant methods, one of skill in the art can obtain gene expression from labelled large nucleic acid molecules delivered into cells at an efficiency that is comparable to that of unlabelled DNA.

Thus, the specification teaches one skilled in the art how to introduce labelled large nucleic acid molecules into cells, how to measure delivery of the labelled nucleic acid molecules to cells by detecting labelled cells and how to measure gene expression from the labelled nucleic acid molecules in cells by detecting the product of a reporter gene.

Knowledge of those of skill in the art

At the time of filing of the application, a broad body of knowledge had amassed in the areas of delivery of nucleic acids to cells and in the use of reporter genes. Numerous such procedures are referenced in the instant application:

Agents to aid in the delivery of nucleic acid molecules and sources therefor are described in the specification. For example, reference is made to electroporation methods and apparatuses *e.g.*, in U.S. Patent Nos. 6,027,488, 5,993,434, 5,944,710, 5,507,724, 5,501,662, 5,389,069, 5,318,515 (page 23, lines 20-26). Procedures and methods for ultrasound systems also are provided. These include systems described in International PCT application No. WO 99/21584 and U.S. Patent No. 5,676,151 (page 22, line 10 through page 23, line 2). Additional delivery agents that can be used in the methods provided in this applicaiton. These include well-known agents, such as cationic reagents

such as Lipofectin, LipofectAMINE, and LipofectAMINE PLUS from Life Technologies, Inc., (Burlington, Ont., *see* U.S. Patent No. 5,334,761 and 5,736,392; *see, also* U.S. Patent No. 6,051,429), Effectene (Qiagen, Inc., Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, Inc., San Luis Obispo, CA) and numerous other cationic lipids (page 20, line 24 through page 21, line 18), as well as non-lipid compounds such as SUPERFECT™ (Activated dendrimer (cationic polymer:charged amino groups); Qiagen, Inc., Mississauga, ON) and CLONfectin™ (Cationic amphiphile N-t-butyl-N'-tetradecyl-3-tetradecyl-aminopropionamidine; Clontech, Palo Alto, CA) (page 21, line 20 through page 22, line 4).

Procedures relating to the use of reporter genes also are referenced and described in the instant application. For example, references are cited for reporters such as fluorescent protein, CAT (chloramphenicol acetyl transferase) (Alton and Vapnek (1979), *Nature 282*: 864-869) luciferase, *e.g.*, firefly luciferase (deWet *et al.* (1987), *Mol. Cell. Biol. 7*: 725-737); bacterial luciferase (Engebrecht and Silverman (1984), *PNAS 1*: 4154-4158; Baldwin *et al.* (1984), *Biochemistry 23*: 3663-3667); and alkaline phosphatase (Toh *et al.* (1989) *Eur. J. Biochem. 182*: 231-238, Hall *et al.* (1983), *J. Mol. Appl. Gen. 2*: 101) (page 16, lines 12-23).

Additionally, agents for detection of labelled nucleic acids such as BrdU-labelled DNA were known. The specification describes such, including, for example, Pittman *et al.*, *J Immunol Methods 103*: 87-92 (1987) (page 28, lines 16-20) and Gratzer *et al.* Cytometry (1981) 6:385-393 (page 55, lines 8-9).

These references to numerous published protocols for the labelling of DNA and detection thereof, agents for delivery of DNA into cells that can be used in the instantly claimed and disclosed methods, and analysis thereof for expression of encoded genes, including reporter gene products, demonstrate the large volume of information regarding tested and reliable agents available at the

time of filing of the instant application and thus evidence the advanced state of the art at the relevant time. The instant application details their application to the claimed and disclosed methods.

Presence of Working Examples

The specification provides numerous working examples and descriptions of the labelling, delivery and detection of delivered nucleic acid, including nucleic acid encoding a reporter gene and the measurement of expression of the reporter gene product (see discussion above). As detailed above, Examples 1, 4, 5, 6, and 7 of the application describe DNA delivery, measurement of labelled cells and measurement of reporter gene activity. Example 1, section B, provides details for the delivery of large nucleic acid molecules (page 40, line 4 through page 41, line 20) and for measuring gene expression from the delivered large nucleic acid molecules (page 41, lines 7-23). Example 4 exemplifies delivery of large labelled DNAs and the detection of labelled cells (page 47, line 22 through page 48, line 4). Example 5 provides detailed methods for the delivery of large nucleic acid molecules and measurement of GFP reporter gene expression from the delivered nucleic acids (page 48, line 15 through page 49, line 31). Example 6 exemplifies the delivery of large nucleic acid molecules to a variety of cell types and the measurement of the encoded GFP reporter gene expression (page 50, line 1 through page 51, line 4). Example 7 provides detailed methods for labeling of large nucleic acids (page 52, lines 12-31), delivery of large labelled nucleic acid molecules to cells using a variety of delivery agents (page 53, line 15 through page 54, line 4 and also Table 1 pages 54-55), the measurement of β-galactosidase activity after delivery (page 54, lines 4-6) and the detection of labeled cells to measure DNA delivery (page 55, line 8 through page 56, line 5 and also Table 2, pages 56-57).

Predictability

As discussed above and as known to those of skill in the art, the level of knowledge and skill in the delivery of DNA and the use of reporter genes for expression in cells as claimed in the instant application was high as of the effective filing date.

The pending claims are directed to methods in which labelled large nucleic acid molecules are delivered to cells, delivery is measured by detecting labelled cells and the detection of a reporter gene product is assessed as a measurement of gene expression. Having described the claimed methods, and detailed the numerous agents and procedures for effecting each step in the methods in the application, there is no issue of predictability in the instant case.

The Office Action alleges that the specification is not enabling for the claimed subject matter because the state of the art of obtaining expression from a labelled nucleic acid molecule at the time the instant application was filed was "highly unpredictable" and Applicant allegedly provides insufficient data to rebut this state of the art. To support the assertion, the Office Action cites Felgner *et al.* ((1999) WO 99/13719), Neves *et al.* (1999/2000) *Bioconjugate Chem.* 11:51-55), and Zelphati *et al.*, (1999) *Hum. Gene Therap.* 10:15-24. Each of these references is discussed in turn below.

<u>Felgner *et al.*</u>

The Office Action alleges that Felgner *et al.* ((1999) WO 99/13719) teaches that standard methods of labeling DNA using fluorescently tagged nucleotides do not allow detection of structurally and functionally intact plasmid in a real-time fashion in viable cells (paragraph bridging pages 1 and 2). The Office Action further points to a passage in Felgner *et al.* that states in its entirety: "All of the technologies discussed above for chemically modifying plasmid DNA result in DNA damage and interfere with its transcriptional activity" (page 2, line 36 through page 3, line 2).

First, it is respectfully submitted that any broad sweeping assertions as to general difficulties of detecting and/or obtaining expression from labelled DNA made in Felgner *et al.*, published in 1999, bears no relevance to the issue of whether the instant application is enabling as of the filing date, March 21, 2001, nor whether the instant application is enabled for the methods it teaches and claims. The citations pointed to by the Office Action only refer to technologies that Felgner *et al.* appraised as of 1999 and only a selected set of methods pertaining to plasmid DNA labeling. Felgner *et al.* does not provide any methods of detecting labelled large nucleic acid molecules, measuring gene expression from labelled large nucleic acid molecules or any limitations pertaining to such methods.

As discussed above, the instant application defines large nucleic acid molecules as being at least about 0.5 megabases in size (*see, e.g.*, page 9, line 30 through page 10, line 16). Felgner *et al.* provides no insights regarding delivery or expression of labelled nucleic acid molecules in this size range. Furthermore, Felgner *et al.* does not have the benefit of the teachings of the instant application

The instant application provides methods for labeling large nucleic acid molecules, delivering the labelled nucleic acid to cells and measuring gene expression as well as delivery (see, for example, Example 7, pages 52-57). The specification further teaches methods of labeling that do not result in the breakdown of the nucleic acids and additionally, the working examples provide a description of reagents that may be used to help stabilize labelled nucleic acids if necessary, such as hexylene glycol, spermine and spermidine (for example, see in Example 7, page 52, lines 29-31). Thus, the specification teaches one of skill in the art how to label large nucleic acids that can be delivered to cells to measure delivery and gene expression and additionally teaches methods to overcome the any limitations described by Felgner *et al.*

Second, the citation referred to by the Examiner from Felgner *et al.* reads in its entirety as:

> Other fluorescent labeling methods which utilize nick translation or photoaffinity labeling result in chemical breakdown of the starting material and thus any observations made with these materials may not be representative of the behavior of the original intact plasmid. None of the technologies presented above allow direct detection of structurally and functionally intact plasmid in a real-time fashion in viable cells (Felgner *et al.*; page 1, line 35 through page 2, line 3).

It is respectfully submitted that this citation has no bearing on the predictability of the methods as instantly claimed for introducing labelled large nucleic acid molecules into a cell and measuring their delivery and encoded gene expression. The methods alluded to by Felgner *et al.* refer to specific methods with specific labels that the author alleges cause breakdown of a particular type of DNA, plasmid DNA. Unlike the instant application, Felgner *et al.* does not provide any teachings for the preparation of labelled large nucleic acid molecules, measuring gene expression or delivery of labelled large nucleic acid molecules or overcoming limitations pertaining to such methods.

<u>Neves *et al.*</u>

The Office Action alleges that Neves *et al.* demonstrates that reporter gene expression was greatly reduced by labelling DNA with *p*-azido-tetrafluorbenzylamido-lissamine and abolished by labelling DNA with rhodamine labelled nucleotides. As discussed above, the assumption by the Examiner that the method of Neves *et al.*, first published in 1999, represents the state of the art of gene expression from labelled DNA as of the filing date of the instant application, March 21, 2001, is unfounded.

Neves *et al.* does not discuss general methods of nucleic acid labeling but in fact uses one specific method of labeling, photo-activated labeling, of a specific type of DNA, plasmid DNA. Further, the authors clearly point out that ultraviolet light used to photo-activate labeling induces DNA damage (page 54, second column, paragraph 3). Neves *et al.* also provides that damaged DNA

such as relaxed or linearized plasmid (page 51, paragraph bridging columns 1 and 2) leading to UV damage of the supercoiled plasmid DNA structure, whereas the instant application teaches methods for measuring gene expression and delivery of large nucleic acid molecules. Neves *et al.* suggests that "inactivation of the plasmid...could be explained by the high number of fluorescent nucleotides incorporated (250 per plasmid molecule) and by the modification of plasmid structure." (Page 54, col. 2, paragraph 4). This statement refers specifically to the the plasmid DNA molecules disclosed in Neves *et al.*, which are small and have distinct structural features relative to large labelled nucleic acid molecules, such as chromosomes, that are elements of the instantly claimed methods.

Neves *et al.*, like Felgner *et al.*, focusses on the limitations of photo-activated labelling of plasmid DNA, not the labelling of large nucleic acid molecules per se. Further, unlike Neves *et al.*, the instant application provides remedies to prevent delivery of damaged DNA and methods to increase nucleic acid stability and intactness (for example, page 33, line 31 through page 34, line 13 and page 52, lines 29-31). The teachings of the instant application recognize the manipulations of DNA that would result in instability or damage, and provide ways to overcome such difficulties. Thus, in addition to the irrelevancy of the general statements in Neves *et al.* to enablement of the instantly claimed methods as of the filing date, the instant application provides remedies for any deficiencies presented by Neves *et al.*.

The Office Action further alleges that Neves *et al.* teaches that the poor expression obtained with labelled vectors might result from interference with the transcription apparatus or sequestration or degradation of the labelled molecules. Again, Applicant respectfully points out that Neves *et al.* does not represent the state of the art as of the filing date of the instant application, nor does it represent a broad treatise of gene expression from labelled DNA.

Further, Neves *et al.* does not have the benefit of the teachings of the instant application.

Neves *et al.* pertains to photo-activated labelling of plasmid DNA of plasmid DNA (page 51, paragraph bridging columns 1 and 2) leading to UV damage of the supercoiled plasmid DNA structure, whereas the instant application teaches methods for measuring gene expression and delivery of large nucleic acid molecules. Neves *et al.* suggests that "inactivation of the plasmid...could be explained by the high number of fluorescent nucleotides incorporated (250 per plasmid molecule) and by the modification of plasmid structure." (Page 54, col. 2, paragraph 4). This statement refers specifically to the the plasmid DNA molecules disclosed in Neves *et al.*, which are small and have distinct structural features relative to large labelled nucleic acid molecules, such as chromosomes, that are elements of the instantly claimed methods.

Similarly, the statement of Neves *et al.* that plasmid-lissamine molecules could be sequestered or degraded in the cytoplasm (page 55, col. 1, first paragraph) has no bearing on the methods of the instant application, which are directed to delivery of labelled large nucleic acid molecules. This statement of Neves *et al.* is in specific reference to microinjecting plasmid-lissamine complexes into the cytoplasm. The instant application teaches numerous delivery agents and delivery methods (page 19, line 8 through page 23, line 26 and see also Examples 4, 5, 6 and 7, pages 46-57) that can be used in a method of delivering labelled large nucleic acid molecules and measuring gene expression and delivery.

<u>Zelphati *et al.*</u>

The Office Action cites Zelphati *et al.* ((1999) *Hum. Gene Therap. 10*: 15-24) as an example of the unpredictability of obtaining expression from a nucleic acid molecule that has been chemically modified to incorporate a label. The Office Action points to a passage in Zelphati *et al.*, which states:

> the methods that have been employed to directly modify DNA either reduce or destroy its ability to be transcribed. In addition, the available approaches to chemically modify plasmid, which utilize photolysis, nick translation, or the use of chemically active nucleotides analogs, attack the DNA randomly so that the final product is chemically heterogeneous and poorly defined.

First, although Zelphati *et al.* points out possible pitfalls of using labelled DNA in obtaining gene expression, these citations from 1999 do not represent the state of the art as of 2001, nor do they suggest that methods of the instant application, filed in 2001, are not enabling.

As with the other citations, Zelphati *et al.* does not have the benefit of the teachings of the instant application. The instant application teaches methods of delivering labelled large nucleic acid molecules and the measurement of gene expression and delivery of these nucleic acids. As discussed above, the large nucleic acid molecules of the instant application are at least about 0.5 megabases in size, and Zelphati *et al.* provides no teaching as to labeling, delivery or expression of nucleic acid molecules in this size range.

Further, the instant application provides ways to overcome the pitfalls referred to in Zelphati *et al.* Specifically, as discussed above, the instant specification teaches one skilled in the art that stability of the nucleic acid molecules to be delivered is assessed, for example by microscopy, after incorporation of label (page 33, line 31 through page 34, line 13); methods are provided for labelling nucleic acid molecules in ways that do not result in instability; and the working examples provide descriptions of reagents that may be used to stabilize labelled nucleic acids such as hexylene glycol, spermine and spermidine (for example see Example 7, page 52, lines 29-31). Thus, the specification teaches one of skill in the art how to make and use the instantly claimed subject matter, including ways to overcome the pitfalls and limitations discussed in Zelphati *et al.*

Second, it is respectfully submitted that a selective reading of Zelphati *et al.*, in which statements regarding the state of nucleic acid labeling and gene expression at the time of filing of the instant application are taken out of context, has resulted in a mischaracterization of the reference. While Zelphati *et al.* may summarize pitfalls of particular techniques as they pertain to plasmid DNAs, the authors then go on to provide a peptide nucleic acid hybridization labelling method for small plasmid DNA molecules that permits gene expression at a level similar to unmodified plasmid DNA.

It appears that the Office Action, in asserting the unpredictability of the art of obtaining gene expression from labelled nucleic acid molecules, has equated "limitations" with "unpredictability." It is respectfully submitted that although certain methods of labeling nucleic acid molecules can be associated with certain limitations in gene expression, this does not establish the art as unpredictable. The issue of whether the specific instant claims are enabled by the specification should not turn on specific limitations previously encountered in measuring delivery and expression of labelled nucleic acids. Rather, the relevant question with regard to enablement of the subject matter of the instant claims is whether the particular steps and materials of the claimed methods are described in the specification in such a way as to enable one skilled in the art to make and use the subject matter **as claimed**. Applicant is not aware of any requirement under current U.S. patent law specifying particular minimum levels of optimization and certified efficacy in order for a treatment-related area of art to qualify as sufficiently "predictable" such that lack of enablement under 35 U.S.C. § 112, first paragraph, is not a consideration. The relevant standard is not that of an established, fully optimized, method; rather, even in an *unpredictable* art, a patent application satisfies the requirements of 35 U.S.C. § 112, first paragraph, as long as it provides sufficient disclosure, either through illustrative examples or terminology, to teach those of skill in the art how to make and use the claimed subject matter without undue, experimentation.

Further, as discussed above, the teachings of the instant application clearly point to the limitations of the art cited by the Examiner, and provide ways to overcome them. Therefore, it is respectfully submitted that even if the art of the use of labelled nucleic acid molecules in delivery and expression systems was not a routine practice at the effective filing date of the subject application and was subject to limitations in its applicability, since the instant application provides solutions to any such pitfalls, such art should not qualify as a major factor in the determination of whether the requirements of 35 U.S.C. § 112, first paragraph, are satisfied with respect to the instantly claimed subject matter.

2. CONCLUSION

In light of the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, the fact that it is predictable to obtain gene expression from labelled nucleic acid molecules, and the breadth of the claims, it would not require undue experimentation for one of skill in the art to follow the steps of the instant methods to introduce labelled large nucleic acid molecules and measure delivery and gene expression thereof in a cell.

Accordingly, a consideration of the factors enumerated in *Ex parte Forman* leads to the conclusion that, based on the disclosure in the specification, undue experimentation would not be required to introduce labelled large nucleic acid molecules into a cell, measure labelled cells as an indication of delivery of nucleic acid and measure the product of a gene encoded by the labelled large nucleic acid as an indication of gene expression in a cell, as instantly claimed.

3. DECLARATION

Notwithstanding the above arguments, to evidence that the methods as claimed operate as claimed, attached is a DECLARATION under 37 C.F.R. §1.132 of Sandra Vanderbyl. The DECLARATION shows that when using the teachings of the application, large labelled nucleic acid is delivered and expressed as claimed.

Also, although this DECLARATION is submitted to rebut the Examiner's assertions of inoperativeness, it also further evidences enablement. It is noted that the level of skill in the biotechnical arts is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as labelling nucleic acids, delivering nucleic acids to cells and measuring delivery of and gene expression from the delivered nucleic acids are known to the skilled artisan.

Although Ms. Vanderbyl is an inventor of this application, in performing the experiments in the DECLARATION, she followed the teachings in the application. Since those of skill in this art typically have advanced degrees, Ms. Vanderbyl, who has an M.S. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol.

The DECLARATION demonstrates that using the procedures as taught in the above-captioned application delivery and expression of labelled large nucleic acid molecules in cells is achieved. The results demonstrate that delivery and expression of labelled large nucleic acid molecules in cells is similar to that of unlabelled nucleic acids.

Specifically, the DECLARATION of Vanderbyl, which incorporates the reference Vanderbyl *et al.*, *Cytometry*, 44:100-105 (2001), demonstrates that large nucleic acid molecules, exemplified using artificial chromosomes, can be (i) labelled with either iododeoxyuridine (IdU) or bromodeoxyuridine (BrdU); (ii) delivered into a host cell by transfection methods using such reagents as

Superfect and LipofectAMINE 2000 (iii) detected by monitoring the labelled cells using an IdU/BrdU antibody and flow cytometry; and (iv) measured for expression of a reporter gene such as green fluorescent protein (GFP) expressed from the labelled nucleic acid molecule in the cell. The DECLARATION further demonstrates that by following the teachings of the specification, one of skill in the art can obtain gene expression of a product encoded by a labelled large nucleic acid at an efficiency that is comparable to that of an unlabelled large nucleic acid under similar conditions.

The DECLARATION describes the labeling of cell lines CHO E42019 and GFP A9, each containing an artificial chromosome of about 120 megabases and 90 megabases, respectively. Labeling is performed with either IdU or with BrdU. Cells are labelled with a range of label concentrations and with continuous and pulse label methods. The DECLARATION demonstrates the use of flow cytometry as well as microscopy to detect labelled cells. Further, the DECLARATION demonstrates that GFP is expressed from cells containing the labelled large nucleic acid, and that this expression (an average efficiency of 47.3%) does not differ significantly from the expression of GFP in unlabeled cells (an average efficiency of 52.8%).

The results of these analyses demonstrate that the methods described in the above-referenced application can be used to monitor labelled large nucleic acid delivery and gene expression of products encoded by the large nucleic acid. Additionally, the DECLARATION demonstrates that in following the steps of the instantly claimed methods as provided in the specification, labeling of large nucleic acids does not affect the expression of encoded gene products as compared to gene expression from unlabelled large nucleic acids.

Therefore, the DECLARATION provides further evidence that the disclosure in the above-captioned application enables methods for delivering labelled large nucleic acid molecules, measuring labelled cells as an indication of delivery of nucleic acid and measuring the product of a reporter gene expressed

from a labelled large nucleic acid as an indication of gene expression in a cell. Vanderbyl *et al.*, hence, is relied upon, not to establish enablement, but to demonstrate that labelled large nucleic acids can be readily made, delivered to cells, detected in cells and expressed in cells by following the teachings in the specification. The DECLARATION demonstrates element-for-element and step-for-step that, by following the teaching in the application, one can: (i) prepare and deliver labelled large nucleic acids to cells; (ii) measure nucleic acid delivery by measuring labelled cells; (iii) measure expression of a gene product encoded by the labelled large nucleic acid; and (iv) obtain gene expression from a labelled large nucleic acid at a level that is comparable to expression from an unlabelled large nucleic acid. Accordingly, Applicant respectfully submits that the claims are commensurate in scope with the Applicant's discovery and its disclosure within the above-captioned application.

THE REJECTION OF CLAIM 9 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claim 9 is rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. Specifically, the Office Action alleges that the reference to "step(a)" in line 1 of the claim has no antecedent basis in Claims 1 and 6, from which Claim 9 depends. Reconsideration of this rejection is respectfully requested in view of the amendments herein and the following remarks.

Claim 9 is amended to more particularly point out the claimed subject matter. Claim 9 is amended to refer to the step of "introducing the labelled large nucleic acid molecules", which finds antecedent basis in Claim 1, upon which Claim 6 and therefore Claim 9 depends. Basis for "introducing labelled large nucleic acid molecules" with a cationic delivery agent as recited in Claim 9 also is found in the instant application (for example, see page 20 line 4 through page 22, line 4 and also in the working examples, Table 1, pages 54-55). Therefore, Claim 9 as amended has antecedent basis in Claims 1 and 6 and is no longer indefinite.

REJECTION OF CLAIMS 1, 2, 4, 6-10, 12, 14-16 and 30 UNDER 35 U.S.C. § 102(b)

A. Claims 1, 2, 4, 6-10, 12, 14-16 and 30 are rejected under 35 U.S.C. § 102(b) as being anticipated by Felgner *et al.* (WO 99/13719 (1999)). The Office Action alleges that Felgner *et al.* discloses a method for detecting or determining delivery and expression of a nucleic acid introduced into a cell comprising: introducing labelled nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and measuring the product of the reporter gene. This rejection is respectfully traversed.

RELEVANT LAW

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S.. 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

"Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter *is* identically disclosed or described in the "'prior art'" . . .the

[r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a 103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a 102, anticipation rejection." (Emphasis in original). *In re Arkey, Eardly, and Long*, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

THE CLAIMS

Claim 1 is directed to a method of nucleic acid delivery and measurement of nucleic acid delivery and gene expression. The steps of the method include:

> introducing labelled large nucleic acid molecules that encode a reporter gene into cells;
> detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and
> measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.

Dependent claims 2, 4, 6-10, 12, 14-16 and 30 specify particulars, such as methods of detection by flow cytometry, fluorometry, cell imaging or fluorescence spectroscopy (claim 2); the type of nucleic acid including DNA (claim 4) artificial chromosomes, plasmids, chromosome fragments, naked DNA or natural chromosomes (claim 12); the types of reporter genes encoding a fluorescent protein or enzyme or antibody (claims 6-8); agents for delivering nucleic acids (claims 9-10); and the types of cells (claims 14-16). Thus, all of the instantly claimed methods are directed to the introduction of labelled large nucleic acid molecules into a cell, detection of their delivery and measurement of expression of a reporter gene encoded by the labelled large nucleic acid molecules.

ANALYSIS

Differences between the claims and the disclosure of Felgner *et al.* (WO 99/13719)

Felgner *et al.* discloses methods for introducing peptide nucleic acid (PNA) plasmid DNA hybrids into cells. The plasmids are on the order of 10 kB, and hence are not large nucleic acids. Felgner *et al.* does not disclose methods for delivery and expression of large nucleic acid molecules.

In the method of Felgner, PNA is hybridized in a sequence-specific manner to the plasmid. In some embodiments of Felgner *et al.*, the PNA is conjugated to a label, exemplified by a rhodamine-PNA conjugate, to monitor plasmid biodistribution. The plasmid encodes a reporter gene, either GFP or β-galactosidase, to measure gene expression. Felgner *et al.* also discloses the use of fluorescence microscopy to monitor labelled cells. Felgner *et al.* discloses the use of cationic liposomes, specifically DOPE:DMRIE, as a delivery agent for plasmid introduction into cells. Felgner *et al.* discloses the use of animal cells such as mouse and monkey cells, specifically mouse melanoma and transformed Cos-7 cell lines, to deliver labelled plasmid DNA.

Felgner *et al.* does not disclose methods for the introduction of labelled large nucleic acids into cells, nor measurement of their delivery or expression of genes encoded by the large labelled nucleic acids.

Analysis

In contrast, claim 1 as amended herein and dependent claims 2, 4, 6-10, 12, 14-16 and 30 are directed to introducing labelled <u>large</u> nucleic acid molecules encoding a gene into cells, and the measurement of nucleic acid delivery and gene expression.

As defined in the specification, large nucleic acids are "at least about 0.5 megabases" (*i.e.*, about 500 kB) (page 9, line 30 through page 10, line 1).

The plasmid DNA molecules diclosed in Felgner *et al.* are of the order of less than 10kB (*see* Example 1 beginning at page 12 and references incorporated therein).

Felgner *et al.* does not disclose methods using labelled large nucleic acids, such as chromosomes, where delivery of the labelled large nucleic acid is detected and expression of genes(s) encoded by the labelled large nucleic acid is measured. All of the rejected claims (Claim 1 and claims dependent thereon), on the other hand, are directed to delivery and expression of labelled large nucleic acids in cells.

Therefore, since anticipation requires that a reference disclose all elements as claimed, Felgner *et al.*, which does not disclose the introduction of a labelled large nucleic acid into a cell, does not anticipate any of the rejected claims 1, 2, 4, 6-10, 12, 14-16 and 30, all of which are directed to methods of introducing labelled large nucleic acids into cells and measuring nucleic acid delivery and gene expression.

B. **Claims 1, 2, 4, 6, 7, 9, 10, 12, 14-16 and 30** are rejected under 35 U.S.C. § 102(b) as being anticipated by Neves *et al.* ((1999/2000) *Bioconjugate Chem.* 11: 51-55) because Neves *et al.* teaches a method for introducing labelled nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid onto a cell; and measuring the product of a reporter gene. This rejection is respectfully traversed.

RELEVANT LAW

See above.

CLAIMS

See above.

ANALYSIS

Differences between the claims and the disclosure of Neves *et al.* ((1999/2000) *Bioconjugate Chem.* *11*: 51-55)

Neves *et al.* discloses the labeling of plasmid DNA with p-azido-tetrafluorobenzylamido-lissamine and with rhodamine nucleotides. Neves *et al.* discloses the introduction of these labelled plasmids and the detection of labelled cells by fluorescence microscopy. Neves *et al.* discloses that their methods of introducing plasmids into cells results in detection of reduced reporter gene (β-galactosidase) activity from labelled DNA as compared with unlabelled DNA (Figure 2, p. 53).

Neves *et al.* does not disclose a method of introducing labelled large nucleic acids into cells, nor measuring delivery of or gene expression from the delivered labelled large nucleic acids. The plasmids labelled by the method of Neves *et al.* are only about 7 kb (*see* Figure 1, page 53), whereas, as discussed above, large nucleic acid molecules as defined in the instant application are "at least about 0.5 megabases" or 500kB (page 9, line 30 through page 10, line 1). Neves *et al.* does not disclose the introduction of labelled nucleic acid molecules that are artificial chromosomes, chromosome fragments or natural chromosomes into cells. Therefore, the plasmids disclosed in Neves *et al.* are several orders of magnitude smaller than the large nucleic acid molecules of the instant application.

Therefore, since anticipation requires that a reference disclose all elements as claimed, Neves *et al.*, which does not disclose the introduction of a labelled large nucleic acid into a cell, nor its detection or measurement of expression of gene(s) encoded by the large nucleic acid, Neves *et al.* does not anticipate any of the rejected claims, all of which specify these elements.

C. **Claims 1, 2, 4, 6, 8-10, 12, 14-16 and 30** are rejected under 35 U.S.C. § 102(b) as being anticipated by Zelphati *et al.* (1999) *Hum. Gene Therap. 10*: 15-24 because Zelphati *et al.* discloses a method for introducing labelled nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid onto a cell; and measuring the product of a reporter gene. This rejection is respectfully traversed.

RELEVANT LAW

See above.

CLAIMS

See above.

ANALYSIS

Differences between the claims and the disclosure of Zelphati *et al.* (1999) *Hum. Gene Therap. 10*: 15-24 ("Zelphati *et al.*")

Zelphati *et al.* discloses a method of hybridizing rhodamine-labelled peptide nucleic acid molecules (PNA) to plasmid DNA encoding a green fluorescent protein (GFP) reporter gene and the visualization of the labelled plasmid and GFP in cells. Zelphati *et al.* does not disclose the introduction of labelled large nucleic acids into cells. The plasmids used in Zelphati *et al.* range in size from about 5 kB to about 8 kB (page 2, column 2, first paragraph). On the other hand, as discussed above, the "large" nucleic acids that are elements of the instant claims are at least about 500 kB in size (page 9, line 30 through page 10, line 1).

Therefore, since anticipation requires that a reference disclose all elements as claimed, Zelphati *et al.*, which does not disclose the introduction of a labelled large nucleic acid into cells, nor the monitoring of nucleic acid delivery and gene expression of products encoded by the labelled large nucleic acid, does not anticipate any of the rejected claims 1, 2, 4, 6-10, 12, 14-16 and 30, all of which specify these elements.

REJECTION OF CLAIMS 1-3 AND 11-13 UNDER 35 U.S.C. §103(a)

Claims 1-3 and 11-13 are rejected under 35 U.S.C. §103(a) as being unpatentable over either one of Felgner *et al.* (WO 99/13719 (1999)) or Zelphati *et al.* (*Hum. Gene Therap. 10*: 15-24 (1999)) in view of Nolan *et al.* (WO 00/34436 (2000)). The Office Actionu alleges that Felgner *et al.* and Zelphati *et al.* teach methods for the detection or determination of delivery and expression of a labelled nucleic acid introduced into a cell including the steps of: introducing labelled nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid into the cell; and measuring the product of the reporter gene.

The Office Action acknowledges that Felgner *et al.* and Zelphati *et al.* do not teach the use of flow cytometry to detect the labelled cells , nor the delivery of large DNA or chromosomes, but urges that Nolan *et al.* cures these deficiencies. The Office Action alleges that Nolan *et al.* teaches a method of delivering DNA into cells wherein cells that have taken up the DNA are detected by flow cytometry. It is further alleged that Nolan *et al.* teaches a method for the delivery of fluorescently labelled large DNA molecules into cells where the large DNA molecule includes natural chromosomes and artificial chromosomes.

With respect to delivery of nucleic acids into cells, the Office Action concludes that it would have been obvious to one of skill in the art to modify the methods taught by Felgner *et al.* and Zelphati *et al.* to include the separation of the labelled cells by flow cytometry as allegedly suggested by Nolan *et al.* The Office Action supports this assertion by stating that one of skill in the art would have been motivated to combine the teachings of Felgner *et al.* or Zelphati *et al.* and those of Nolan *et al.*, because Nolan *et al.* states that "flow cytometry methods such as fluorescence-activated cell sorting (FACS) are ideal tools to employ in chromosome insertion methods due to their ability to rapidly process and analyze large numbers of individual cells"; therefore, absent evidence to the contrary, one of skill in the art would have had a reasonable

expectation of success in using the methods of Nolan *et al.* to deliver any fluorescently labelled nucleic acid.

With respect to obtaining gene expression from labelled DNA introduced into cells, the Office Action concludes that it would have been obvious to one of skill in the art to modify the methods taught by Felgner *et al.* and Zelphati *et al.* according to the teachings of Nolan *et al.* to deliver fluorescently labelled transcriptionally active chromosomes into cells. The Office Action supports this assertion by stating that one of skill in the art would have been motivated to combine the teachings of Felgner *et al.* or Zelphati *et al.* and those of Nolan *et al.* because Nolan *et al.* states that "the introduction of intact single chromosomes into cells offers unprecedented usefulness as a...method for generating transgenic animals" and Zelphati *et al.* teaches that their methodology provides a means to tag DNA without disrupting the structural or functional integrity of the DNA. Therefore, it is alleged that absent evidence to the contrary, one of skill in the art would have had a reasonable expectation of success in using the labeling methods of Felgner *et al.* and Zelphati *et al.*. to deliver any nucleic acid molecule.

This rejection is respectfully traversed.

RELEVANT LAW

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." In re Keller, 642 F.2d 413, 425,

208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

CLAIMS

As described above, claims 1-3 and 11-13 are directed to methods of introducing labelled large nucleic acids encoding a reporter gene into cells and measuring DNA delivery and reporter gene expression. Claims 2 and 3 are directed to the use of flow cytometry, fluorometry, cell imaging or fluorescence spectroscopy in detecting labelled cells. Claims 11-13 further define the large nucleic acid as natural chromosomes, artificial chromosomes, plasmids, fragments of chromosomes and naked DNA greater than about 0.6 megabases in size.

Teachings of the cited art and the differences between the teachings of the cited art and claims 1-3 and 11-13

Felgner *et al.*

As discussed above, Felgner *et al.* teaches methods for introducing peptide nucleic acid (PNA), including rhodamine-PNA conjugates, hybridized in a sequence-specific manner to plasmid DNA into cells and measuring gene expression from these plasmids.

Felgner *et al.* does not teach or suggest methods for the delivery of large labelled nucleic acid molecules. As discussed above, the instant application

defines "large" nucleic acids as being "at least about 0.5 megabases" (*i.e.*, about 500 kB) (page 9, line 30 through page 10, line 1).

The plasmid DNA molecules taught by Felgner *et al.* are of the order of less than 10kB (*see* Example 1 beginning at page 12 and references incorporated therein). Felgner *et al.* does not teach any methods using labelled large nucleic acids, such as chromosomes, where delivery of the labelled large nucleic acid AND expression of genes(s) encoded by the labelled large nucleic acid are measured. Felgner *et al.* does not teach the introduction of labelled artificial chromosomes, chromosomes fragments or natural chromosomes into cells.

Zelphati *et al.*

As discussed above, Zelphati *et al.* teaches methods for hybridizing rhodamine-labelled peptide nucleic acid (PNA) to plasmid DNA encoding a reporter gene and the detection of the labelled plasmid and reporter gene products (GFP or β-galactosidase) in cells.

Zelphati *et al.* does not teach or suggest the introduction of large nucleic acids nor the introduction of large labelled nucleic acids. The plasmids used in Zelphati *et al.* range between about 5 kB and about 8 kB (page 2, column 2 first paragraph). In contrast, the large labelled nucleic acids used in the instantly claimed methods are at least about 500 kB (page 9, line 30 through page 10, line 1). Zelphati *et al.* does not teach the introduction of labelled artificial chromosomes, chromosome fragments or natural chromosomes into cells. Further, Zelphati *et al.* does not teach the introduction of labelled nucleic acids that are plasmids or naked DNA that are large nucleic acids, at least about 0.5 megabases (500kB) as defined by the instant application. As mentioned above, the plasmid DNA taught by Zelphati *et al.* is at least about two orders of magnitude smaller than the nucleic acids that are delivered by the instantly claimed methods.

Nolan *et al.*

Nolan *et al.* teaches methods and an apparatus that employ fluorescence activated cells sorting (FACS) to process large numbers of cells in a chromosome insertion protocol. Nolan *et al.* teaches subjecting a cell to a laser pulse light under conditions sufficient to form a transient hole in the cell plasma membrane, then introducing a single chromosome into the cell through the hole. Nolan *et al.* further teaches that fluorescence activated cells sorting (FACS), a type of flow cytometry, can be employed to confirm the insertion of a single chromosome within a cell.

Nolan *et al.* does not teach or suggest the introduction of labelled large nucleic acid molecules encoding a reporter gene into cells, nor measurement of reporter gene product as an indication of gene expression. The methods of Nolan *et al.* are focussed on the verification of single chromosome insertion. Nolan *et al.* mentions that the chromosome may be fluorescently labelled, but provides no teaching or suggestion for obtaining such labelled chromosomes. Further, Nolan *et al.* does not teach the detection of reporter gene expression in combination with measurement of nucleic acid delivery of labelled large nucleic acid molecules encoding the reporter gene.

ANALYSIS

The combination of teachings of Felgner *et al.* or Zelphati *et al.* with the teachings of Nolan *et al.* does not result in the instantly claimed methods.

None of the cited references, singly or in any combination, teaches or suggests a method for the introduction of labelled large nucleic acid molecules encoding a reporter gene and the detection of delivery and measurement of gene expression. As discussed above, neither Felgner *et al.* nor Zelphati *et al.* teaches or suggests methods for introducing labelled large nucleic acid molecules into cells and detecting their delivery and measuring their expression.

The methods of Felgner *et al.* and Zelphati *et al.* are directed to the monitoring delivery and expression of plasmid DNA that is under 10 kB in size.

Nolan *et al.*, which is directed to the insertion of chromosomes into cells, provides no teaching or suggestion regarding any method for delivery of large nucleic acid molecules nor for detecting or determining delivery and expression of such nucleic acid. Nolan *et al.* only provides an apparatus to deliver chromosomes into cells and a method to screen for and recover eukaryotic cells having a single chromosome inserted therein. Nolan *et al.* does not teach or suggest monitoring delivery and measuring expression of chromosomes in the cells. Therefore, Nolan *et al.* does not cure the deficiencies of either Felgner *et al.* or Zelphati *et al.*

The Office Action alleges that absent evidence to the contrary, one of ordinary skill in the art would have had a reasonable expectation of success in using the methods of Nolan *et al.* in combination with either Felgner *et al.* or Zelphati *et al.* to deliver <u>any</u> fluorescently labelled nucleic acid.

The claims of the present method are directed to steps of: introducing labelled large nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid into the cell; and measuring the product of the reporter gene. The combination of teachings of tghe references cited must provide all of these steps, not just the single step of introducing fluorescently labelled nucleic acid. Felgner *et al.* and Zelphati *et al.* do not provide any teaching or suggestion for delivery of large nucleic acids molecules nor monitoring/measuring delivery and expression of large labelled nucleic acids in cells. Nolan *et al.* is directed to single chromosome insertion and cell sorting to recover cells containing inserted chromosomes. Therefore, Nolan *et al.*, which also does not teach or suggest a method for monitoring/measuring delivery and expression of large labelled nucleic acids in cells, cannot cure the deficiencies of either Felgner *et al.* or Zelphati *et al.*

One of ordinary skill in the art would have no expectation of success from the combination of Felgner *et al.* or Zelphati *et al.* with Nolan *et al.* in measuring

the delivery and expression of labelled large nucleic acids encoding a reporter gene, *i.e.*, to provide all the steps of the instantly claimed methods. The Examiner asserts that one of ordinary skill in the art could use the delivery method of Nolan *et al.* in the method of Felgner *et al.* or Zelphati *et al.*; Nolan *et al.* does not, however, provide methods for the measurement of nucleic acid delivery and gene expression, nor any teaching or suggestion thereto. In fact, insertion of a single copy of fluorescently labelled chromosome as taught by Nolan et al. would be incompatible with the methods of either Zelphati *et al.* or Felgner *et al.*, which each teach delivery of several thousand copies of plasmid molecules to a cell in order to measure their delivery and expression.

For example, Zelphati *et al.* (which is a near identical if not identical method to that of Felgner *et al.*) states that an average of 30,000-50,000 copies of plasmid/cell were present using the method described therein (page 19, column 2, paragraph 2). There is no teaching or suggestion that the method of Zelphati *et al.*, designed to operate with many thousands of copies of plasmid to monitor gene expression, would be useful or workable with a lower number of nucleic acid molecules, such as the introduction of a single fluorescent chromosome into a cell as taught by Nolan *et al.* Neither the method of Felgner *et al.* nor that of Zelphati *et al.* teaches or suggests measurement of gene expression from such a low copy number of nucleic acid. Thus, one of ordinary skill in the art would have no reasonable expectation of success in combining the delivery methods of Nolan *et al.* with the teachings of Felgner *et al.* or Zelphati *et al.* to obtain and measure delivery and expression of a labelled large nucleic acid encoding a reporter gene.

The Office Action further alleges that it would have been obvious to one of skill in the art to modify the methods taught by Felgner *et al.* and Zelphati *et al.* according to the teachings of Nolan *et al.* to deliver fluorescently labelled transcriptionally active chromosomes into cells. The Office Action alleges that one of skill in the art would have been motivated to combine the teachings of

Felgner *et al.* or Zelphati *et al.* and those of Nolan *et al.* because Nolan *et al.* states that "the introduction of intact single chromosomes into cells offers unprecedented usefulness as a...method for generating transgenic animals" and Zelphati *et al.* teaches that their methodology provides a means to tag DNA without disrupting the structural or functional integrity of the DNA.

None of the cited references, singly or in any combination, teaches or suggests a method for the introduction of labelled large nucleic acid molecules and the determination or detection of delivery and gene expression where the introduced nucleic acid is a large nucleic acid or a chromosome as defined herein (about 0.5 megabases or greater, *see, e.g.*, page 9, line 30 through page 10, line 16). The Examiner asserts that the motivation to combine Zelphati *et al.* or Felgner *et al.* with Nolan *et al.* comes from a citation, quoted in the paragraph above, that is in fact a general statement on the utility of introducing chromosomes into cells; Nolan *et al.* does not teach or suggest anything about monitoring delivery of chromosomes and gene expression from chromosomes. Additionally, Nolan *et al.* suggests "there exists a need for a method to rapidly and reliably process cells in a manner that provides for the introduction of a single chromosome into a cell in a verifiable manner" (page 1, lines 25-27). Nolan *et al.* provides no teaching or suggestion that there is a need for monitoring gene expression nor measuring gene expression along with DNA delivery as set forth in the instantly claimed methods. Thus, contrary to the Examiner's assertion, there is no teaching or suggestion in Nolan *et al.*, which lacks any reference to measuring gene expression, that would cure the deficiencies in Felgner *et al.* or Zelphati *et al.*, neither of which provide any teaching or suggestion as to obtaining successful delivery and gene expression of large nucleic acid molecules.

The Office Action further alleges that absent evidence to the contrary, one of ordinary skill in the art would have had a reasonable expectation of

success in using the labeling methods of Felgner *et al.* and Zelphati *et al.* in combination with Nolan *et al.* to deliver any nucleic acid molecule.

The methods of Felgner *et al.* and Zelphati *et al.* provide only for labelling of small plasmid DNAs with peptide nucleic acids (PNAs). There is no teaching or suggestion in Felger *et al.* or Zelphati *et al.* that the labeling method is suitable for large nucleic acids or how PNA may be used with large nucleic acid molecules. Both references use plasmids on average 6-7 kilobases (no larger than 8.2 kilobases). "Large" nucleic acid as defined in the instant application is at least about 0.5 megabases (500 kilobases), at least two orders of magnitude larger than what is taught by Felgner *et al.* and Zelphati *et al.* Nolan *et al.* does not cure this deficiency, providing only that labelled chromosomes can be used with the described FACS apparatus. Nolan *et al.* does not teach or suggest how one obtains labelled large nucleic acids. It is only in the instant application that methods are provided for labeling large nucleic acid molecules such that the labelled large nucleic acids encoding a reporter gene can be delivered and expressed in cells with an efficiency comparable to that of unlabelled nucleic acids.

Furthermore, the labelling methods taught by Felgner *et al.* and Zelphati *et al.* are specially engineered to maintain the structural integrity of <u>plasmid</u> DNA. Neither Zelphati *et al.* nor Felgner *et al.* teaches or suggests methods for the labeling of large nucleic acids in a manner that maintains their transcriptional integrity. The methods taught by Felgner *et al.* and Zelphati *et al.* rely on sequence-specific hybridization of a peptide nucleic acid (PNA) with a plasmid to conjugate the label to the plasmid. Neither reference provides guidance on how one of ordinary skill in the art would engineer such conjugates in large nucleic acids, given the limitations described by both Felgner *et al.* and Zelphati *et al.*, for example, that PNAs can inhibit gene expression, block restriction enzyme activity and act as an artificial promoter (Felgner *et al.*, page 2, lines 21-25) and furthermore, in designing PNAs that "the probe itself should not have influence

on plasmid function" (Felgner *et al.*, page 2, lines 35-36). Additionally, Zelphati *et al.* states that PNAs are known to inhibit several metabolic processes such as translation, including ribosome translocation, transcription, including inhibition of RNA polymerase and telomerase activity (page 20, column 2, paragraph 3). Neither Felgner *et al.* nor Zelphati *et al.* teach or suggest how to overcome these limitations for the labeling of large nucleic acids such that function is not impaired or altered as a result of the PNA conjugation. Nolan *et al.*, which offers no teachings as to labelling large nucleic acids, much less labelling in a manner that preserves their transcriptional integrity, does not remedy the deficiencies of Felgner *et al.* and Zelphati *et al.* It is only in the instant application that a method is provided in which labelled large nucleic acids encoding a reporter gene that maintain their transcriptional integrity are introduced into cells, and their delivery and expression measured.

The rejection over the cited references is based on improper use of hindsight

The disclosure of the Applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. *In re Laskowski*, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983). It appears that the Examiner has combined the teachings of the cited art with those of the instant application.

Since the cited references do not teach or suggest any of the elements of the instantly claimed methods pointed out by the Examiner (introducing labelled large nucleic acid molecules encoding a reporter gene into a cell, detecting labelled cells as an indication of nucleic acid delivery, and measuring the product

of the reporter gene as an indication of DNA expression in the cell), as set forth in the instantly claimed methods, the only way to combine the references to result in the instantly claimed methods would require the use of the instant application as a guide to look for isolated elements of, *e.g.*, "DNA", "chromosome", "labeling", and "gene expression" and combine them to produce the instant claims. None of these elements are recited in the cited references in the context of obtaining and introducing labelled large nucleic acids into cells and measuring their delivery and expression. In fact, the only reference that mentions a "large" nucleic acid (chromosome), Nolan *et al.*, is not directed to any method of nucleic acid delivery and expression in cells at all; rather, it is aimed solely at inserting a single chromosome into cells, and cell-sorting (FACS) to retrieve cells containing a single inserted chromosome. Any labeling of the chromosome taught in Nolan *et al.* is in the context of cell-sorting, not to monitor delivery and expression. Therefore, it appears that the Examiner has isolated these elements from the cited references and incorporated them into the methods as instantly claimed.

Therefore, in addition to the fact that the combination of the cited references does not result in the instantly claimed subject matter, the Office has improperly employed hindsight in setting forth an alleged *prima facie* case of obviousness.

CONCLUSION

In summary, the combination of the teachings of Felgner *et al.* or Zelphati *et al.* with Nolan *et al.* does not result in the instantly claimed subject matter, the methods of which include steps of: introducing labelled large nucleic acid molecules that encode a reporter gene into cells; detecting labelled cells as an indication of delivery of the nucleic acid into the cell; and measuring the product of the reporter gene. None of the references, singly or in any combination, teaches or suggests a method for detecting delivery and gene expression in cells that involves introducing labelled large nucleic acids encoding reporter genes

into cells, measuring labelled cells as an indication of delivery, and measuring reporter gene expression as an indication of DNA expression. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: DE JONG et al.

Serial No.:09/815,981 Group Art Unit: 1636

Filed: March 22, 2001 Examiner: Sullivan, Daniel M.

For: METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF

ATTACHMENTS

The following attachments are provided in connection with the Amendment filed herewith.

1. Marked up paragraph and claims pursuant to 37 C.F.R. §1.121.
2. A DECLARATION of VANDERBYL pursuant to 37 C.F.R. §1.132.
3. A copy of the following publication:

   Vanderbyl *et al.* (2001) *Cytometry 44*:100-105

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: de Jong *et al.*
Serial No.: 09/815,981
Filed: March 22, 2001
Conf. No.: 7622
Cust. No.: 24961
For: *METHODS FOR DELIVERING NUCLEIC ACID MOLECULES INTO CELLS AND ASSESSMENT THEREOF*
Art Unit: 1636
Examiner: Sullivan, D.M.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
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Date of Deposit June 16, 2003

I hereby certify that this paper and the attached papers are being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and addressed to:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

06/16/2003 Debra Walker
Date Debra Walker

MARKED UP PARAGRAPHS AND CLAIMS (37 C.F.R. § 1.121)

IN THE SPECIFICATION:

Please amend the paragraph on page 1, lines 14-23, with the following:

A number of methods of delivering nucleic acid molecules, particularly plasmid DNA and other small fragments of nucleic acid, into cells have been developed. These methods are not ideal for delivery of larger nucleic acid molecules. Thus, there is a need for methods of delivering nucleic acid molecules of increasing size and complexity, such as artificial chromosomes, into cells. Methods are required for use with *in vitro* and *in vivo* procedures such as gene therapy and for production of transgenic animals and [pants] plants. Furthermore, there is a need for the ability to rapidly and simply determine and assess the efficiency of delivery of DNA into cells.

IN THE CLAIMS:

Please amend claims 1, 3, 10, 16 and 30 as follows:

1. (Amended) A method for detecting or determining delivery and expression of a nucleic acid introduced into a cell [comprising;]comprising:

introducing labelled large nucleic acid molecules that encode a reporter gene into cells;

detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and

measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.

3. (Amended) The method of claim 1, wherein the labelled cells are detected by flow [cytometry]cytometry.

9. [(Twice Amended)] (Thrice Amended) The method of claim 6, wherein [step (a)] the step of introducing labelled large nucleic acid molecules comprises contacting the nucleic acid [molecule] molecules with a delivery agent that comprises a cationic compound.

10. (Twice Amended) The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), [$C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2.4CF_3CO_2H$, $C_{40}H_{84}NO_3P.CF_3CO_2H$, $C_{50}H_{103}N_7O_3.4CF_3CO_2H$, $C_{55}H_{116}N_8O_2.6CF_3CO_2H$, $C_{49}H_{102}N_6O_3.4CF_3CO_2H$, $C_{44}H_{89}N_5O_3.2CF_3CO_2H$, $C_{41}H_{78}NO_8P$), $C_{100}H_{206}N_{12}O_4S_2.8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9.13CF_3CO_2H$, $C_{43}H_{88}N_4O_2.2CF_3CO_2H$, $C_{43}H_{88}N_4O_3.2CF_3CO_2H$]$C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \bullet 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \bullet CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \bullet 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \bullet 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \bullet 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \bullet 2CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{100}H_{206}N_{12}O_4S_26 \bullet 8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9 \bullet 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \bullet 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \bullet 2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

16. (Twice Amended) The method of claim 14, wherein the [cells,]cells are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

30. (Amended) The method of claim 1, wherein the cell is selected from the group consisting of a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed [cells]cell and a tumor cell.